POLYCYSTIC LIVER DISEASE

Clinical characterisation and evidence based treatment development

Hedwig M.A. D'Agnolo

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Chapter 1

General introduction and outline of this thesis

INTRODUCTION

Polycystic liver disease

Polycystic liver diseases (PLD) are a group of genetic disorders characterized by the progressive formation of hepatic cysts. [1] PLD is present in two genetically distinct disorders; as the primary phenotype in autosomal dominant PLD (ADPLD); and secondary to renal cysts in autosomal dominant polycystic kidney disease (ADPKD). In this thesis I focus on the phenotype and treatment of PLD in both disorders.

ADPLD is a rare disease and a prevalence of 1: 158.000 has been estimated in the Netherlands.[2] Mutations in *PRKCSH*, *Sec63* or *LRP5* are associated with ADPLD, though collectively they only account for approximately 25% of the cases.

ADPKD is the most common inherited renal disease (prevalence of 1:400-1:1000) and is characterized by the development of renal cysts and a variety of extra-renal manifestations.[3] Progressive kidney cyst growth leads to end-stage renal disease in a large proportion of patients, typically after the fourth decade of life. [3-5] Liver cysts are the most common extra-renal phenotype in ADPKD, present in 94% of the population older than 35 years. [6] In contrast to ADPLD, almost 100% of cases can be explained by mutations in known genes. *PKD1* or *PKD2* mutations are identified in respectively 80-85% and 15-20% of the ADPKD population. [4]

Pathophysiology

Several signal transduction pathways are activated in polycystic livers regulating cyst growth. The *PKD* genes involved in this process encode for polycystin 1 and 2. These are integral membrane proteins acting as a Ca^{2+} permeable receptor channel complex. Mutations in polycystins result in decreased intracellular Ca^{2+} levels and subsequent increased intracellular cyclic adenosine monophosphate (cAMP) levels. This leads to cholangiocyte hyperproliferation, enhanced fluid secretion and eventually progressive cyst formation and growth. [1,7]

Diagnosis

Diagnosis of PLD can be made by radiological imaging such as ultrasonography, CT or MRI scan.[1] There is no strict definition for PLD. A cut-off value of ≥ 20 liver cysts is often used in literature because we assume that the presence of ≥ 20 cysts is due to ADPLD or ADPKD, instead of sporadic cysts.

For the diagnosis of ADPKD in individuals with a known family history of ADPKD, diagnosis is based on modified Ravine criteria which includes age and number of cysts. [8] In absence of a family history of ADPKD, a presumptive diagnosis can be made when >10 cysts in each kidney are present and other renal diseases are absent. [9]

Both ADPLD and ADPKD can be confirmed by genetic testing. As indicated, only a minority of ADPLD patients have a genetic mutation. Hence, genetic testing is not part of clinical practice because of technical challenges and high costs of DNA analysis. Therefore, diagnostic testing is restricted to cases with equivocal or atypical renal imaging findings.[5]

Disease severity

Liver phenotype, in terms of number, size and spread of cysts throughout the liver is an important indicator used to determine treatment strategy. Classification of liver phenotype by Gigot, Schnelldorfer or Qian's categorization might help to choose the right treatment strategy for individual patients. [10-12] However, categorization of patients according to these classifications is not standard of care.

A normal liver weighs approximately 1500mL. [13] Presence of PLD can drive liver volume to >10L in some patients. [1] Disease severity can be assessed by measuring liver volume on CT or MRI. A common method for volumetry is segmentation which includes the manual delineation of transversal CT or MRI images followed by interpolation of slices by an automatic algorithm. [14]

Clinical characterization

The natural course of PLD varies extremely among individual patients. Some patients develop symptomatic hepatomegaly, while others do not. Liver growth in PLD is estimated to be between 0.9-3.2% per year, based on results of placebo groups in clinical trials. [15-17] Of note, clinical trials have also shown that some patients have spontaneous reduction of liver volume while others have extreme increases. [17]

Progressive cyst growth has been associated with several risk factors. Previous cohort studies showed that the prevalence of liver cysts increases with age and that females have a higher risk to develop severe PLD. [2,6,18] In 1997, a prospective clinical trial treating postmenopausal women with estrogen, showed a significant increase in liver volume after treatment compared to no treatment. Prior pregnancies were a strong predictor of presence of hepatic cysts. [19] However, this study included only 19 patients and these results have not been replicated since. [19] A recent individual pooled data analysis found that especially young females (≤48 years) demonstrate progressive cyst growth, compared to older women and men. [20] These results might implicate an important role for female hormones in biogenesis of hepatomegaly. Finally, there is no evidence that ADPKD genotype is related to PLD phenotype. [21] For ADPLD, a genetic mutation in *sec63* or *PRKCSH* was associated with symptomatic disease in a cross sectional analysis of 137 patients, although liver volume was not included in this analysis and no conclusions can be drawn about the association with severity of PLD in terms of volume. [2]
A small proportion of patients with progressive cyst growth and massive hepatomegaly, may develop symptoms and experience a reduced health-related quality of life. [22] Common symptoms are early satiety, abdominal distension and dyspnea. [14] For ADPKD patients, pain is assumed to be an important symptom and this might be related to kidney volume. [23] However,

the exact relation of liver and kidney volume with symptom burden or health-related quality of life is not yet elucidated.

Due to the low prevalence of PLD, physicians are not exposed to large patients groups. Therefore, their knowledge on clinical course, treatment efficacy and prognosis can be limited. These difficulties impede research in mapping natural course of PLD. A registry would be useful to create a large cohort of patients in order to fill in these gaps in knowledge.

Therapy

Current therapies

Therapy is indicated in patients who suffer from symptomatic hepatomegaly. A clear definition of symptomatic hepatomegaly is lacking, as well as standards that guide us when to initiate therapy and which therapy to give to individual PLD patients. [24] As a result, there is a variability in treatment between patients in clinical practice.

Current therapies can be divided in experimental and non-experimental therapies. Non-experimental therapies include a radiological therapy, aspiration sclerotherapy, and several surgical procedures such as fenestration, resection and liver transplantation. Aspiration sclerotherapy involves radiological cyst aspiration followed by administration of a sclerosing agent to destruct cyst wall. [1,25] Patients with 1 or 2 large (>5cm) symptomatic dominant cysts are good candidates for this procedure. Fenestration is a suitable therapy for patients with large (>10cm) cysts or multiple medium-sized cysts with large areas of non-cystic liver parenchyma. This is a surgical procedure that combines aspiration with deroofing of cysts. The advantage

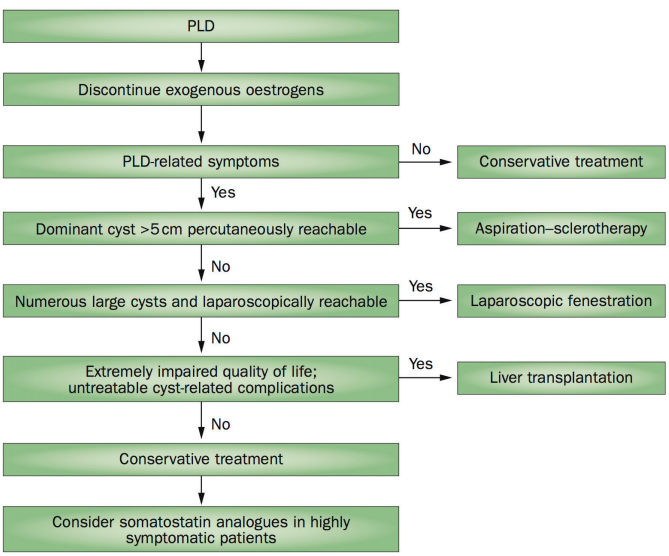

Figure 1. Flowdiagram for treatment strategy in PLD patients 1 [with author permission]

of this method beyond aspiration sclerotherapy is the possibility to treat multiple cysts in a single procedure. Hepatic resection can be applied to patients with livers that have at least one segment with predominantly normal liver parenchyma. This procedure is not often performed due to the rarely present phenotype and the high complication rate. The only curative therapy is liver transplantation, though in terms of risk and scarcity of donors this treatment strategy is not widely applied.[1,25] Figure 1. shows a flow-diagram that might facilitate the choice of therapy.[1]

Experimental therapies

Most symptomatic patients do not fulfill the criteria for the above mentioned therapies. Conservative treatment entails pain medication and life style changes, though there is no solid evidence for these strategies. The risk of complications or recurrence with invasive therapies is high, and with the exception of liver transplantations they do not change natural course. This emphasizes the need for an effective, non-invasive therapy.

Stimulation of cAMP results in cholangiocyte proliferation, and the discovery of this pathway has led to the development of novel therapeutic approaches for treatment of PLD. Pharmacological inhibition of this signaling pathway by somatostatin analogues, has shown to be successful in reducing liver volume up to 5% after administration for 6-12 months. [15,26,27] Octreotide and lanreotide both bind to somatostatin receptors expressed in cyst epithelium. These drugs reduce intracellular levels of cAMP and inhibit cholangiocyte proliferation and cyst fluid accumulation.[28,29] Unfortunately, drawbacks of somatostatin analogues are side effects, patients unresponsiveness and costs. This highlights the need for other options. Ursodeoxycholic acid (UDCA) reduces cystogenesis by both increasing Ca^{2+} and decreasing cAMP levels in cystic cholangiocytes. Experimental evidence has shown that UDCA was able to inhibit proliferation of human cholangiocytes in vitro and hepatic cystogenesis in PCK rats, an animal model of PLD. [30,31]

In conclusion, there are gaps in our understanding of the clinical profile of PLD and the response to various treatment options. The ultimate goal would be to develop an evidence-based guideline on whom to treat, what to treat, when to initiate therapy and which therapy to give. We have investigated a number of specific objectives that will contribute to achieve this goal.

First, we need to elucidate the role of liver and kidney volume on symptom burden. We also need to gain more insight in determinants associated with severe PLD to create risk groups. Second, we need to identify factors that play a role in the treatment decision process. Finally, we need to find out whether UDCA is a successful non-invasive therapy for PLD.

The aim of this thesis is to explore who is in need of therapy, who receives therapy and whether treatment with UDCA is an effective therapy for PLD.

To address these questions, we have structured this thesis in three parts. For each part the hypothesis and rationale, research model and research questions will be presented.

1. Who should we treat and what should we target?

The main indication to initiate treatment in PLD patients is symptomatic hepatomegaly or severe PLD. The question is who develops severe PLD and who will be in need of treatment? Former studies have found evidence among patient-related risk factors for severe PLD such as age, female gender, estrogen use and pregnancies. [2,6,18,19] The effect of ADPLD genotype on PLD phenotype remains unclear. Recently, a cross-sectional study investigated the role of ADPKD genotype on PLD phenotype and failed to detect an association. The question is whether genotype-phenotype associations should be studied in a cross sectional manner. Our editorial gives an overview of the current knowledge among determinants associated with severe PLD **(chapter 2)**. We used this to share our thoughts about future steps that are required to understand the relation between genotype and phenotype.

When we know the population at risk for symptomatic hepatomegaly we need to discover what we should treat in order to prevent or reduce symptoms. Current and experimental therapies for severe PLD focus on reducing liver volume in order to diminish symptoms. [1] However, the role of liver volume in relation to symptom burden is not well understood. There is limited knowledge on the role of other factors such as medical history, in symptom burden. As a consequence, we do not know what and how strong the effect of volume reduction will be on symptom burden. This thwarts the understanding of the population who is most likely to benefit from treatment. In addition, the majority of PLD patients possesses renal cysts as well (ADPKD) which complicates the understanding of the relation between liver volume and symptom burden. Multiple studies have already tried to separate the effect of kidney and liver volume on symptom burden, but results are conflicting. [22,23,32,33] We hypothesized that in patients with ADPKD a combination of liver and kidney volume is a better predictor for symptom burden than liver or kidney volume alone. We also think that sex might be a confounder as women have a higher risk to develop hepatomegaly. If both renal and liver volume play a role in symptom burden, treatment should target both. A difference in association between volume and symptom burden between men and women would result in different treatment strategies for both sexes.

We chose to perform a cross-sectional analysis of patients with later stage ADPKD **(chapter 3)**. We used baseline data of patients included in a multi-center, randomized, controlled clinical trial executed in the Netherlands. The advantage of this study model was the protocolised character of data collection. The selection of later stage ADPKD patients ensured that this population had a higher risk to be symptomatic and to have enlarged kidneys and livers. This facilitates to study the relation between organ enlargement and symptoms. We composed the following research questions:

- What are risk factors for severe PLD?
- What is the effect of ADPKD genotype on PLD phenotype?
- What genotype-to-phenotype concepts should be studied to elucidate whether genotype influences phenotype variability?
- What are the most common gastro-intestinal symptoms in ADPKD patients?
- How many patients experience pain?
- What is the relation of kidney and liver volume with ADPKD-related symptom burden?
- Is the combination of kidney and liver volume stronger associated with ADPKD-related symptom burden than kidney and liver volume alone?
- What is the role of sex in the relation between kidney liver volume and symptom burden?

2. Who do we treat?

There are no clear guidelines for treatment of PLD, so who should actually be treated? Getting more insight in patients and disease related factors involved in treatment decision might help to find determinants associated with a more severe disease course. It also gives physicians insight in the process of treatment decision. We hypothesize that females have a higher likelihood to receive treatment, due to a higher risk of hepatomegaly. We also think that in the absence of clear guidelines for initiating therapy in PLD, the decision to treat depends on expertise and opportunities of physicians.

To establish a clinical characterisation of the population that we treat, a registry design was used to create a cohort of PLD patients. A registry is an excellent observational study method to collect clinical data from individual patients to study the clinical course of a rare disease, such as PLD. Prior to the development of our registry we studied literature to find out what fundamental aspects should be included in our database. We also interviewed several researchers deemed experts in the field of registry studies. We shared our experiences through a paper that reflects our thinking how to build a successful registry **(chapter 4)**. The registry we created was an international database including PLD patients from two nationwide referral centers in Belgium and the Netherlands. We were able to perform a cross-sectional analysis on the role of patients characteristics, disease specific factors and center on treatment decision **(chapter 5)**. Our registry paper and cross-sectional analysis will help to answer the following research questions:

- What are the main aspects of a clinical registry?
- Which properties ensure a successful registry?
- How many patients included in our registry receive invasive treatment for PLD?
- Which patient characteristics and disease factors are associated with invasive treatment?
- What is the role of center in treatment decision and the choice for a specific therapy?
- Which determinants are associated with specific invasive treatment modalities?

3. A novel therapy for PLD?

Current therapies for PLD are mainly invasive, have high complication rates while the chance of success is difficult to predict. In clinical practice, a large group of patients is symptomatic while liver is moderately enlarged. In those cases, invasive therapy is often a step too far. Therefore we need a non-invasive treatment for PLD. An animal model of rats suffering from polycystic livers has shown that UDCA inhibits proliferation of polycystic human cholangiocytes in vitro and hepatic cystogenesis in vivo.[31] Therefore, we hypothesized that UDCA was effective in reducing TLV, liver cyst volume and symptoms in PLD patients.

The best way to assess the efficacy of UDCA on reducing TLV in PLD was to design a randomized controlled trial **(chapter 6a)**. As we learned from our registry experiences, international collaboration is essential in the field of rare diseases. Therefore we designed this trial in collaboration with Spain and created a multicenter, international trial including three tertiary centers for PLD. Collaboration with tertiary centers facilitates identification of patients with symptomatic hepatomegaly, the study population that is in need of therapy. This randomized controlled trial will help to answer the following research questions **(chapter 6b)**:

- Does UDCA affect TLV in PLD?
- What is the effect of UDCA on symptoms in PLD?
- What is the effect of UDCA on health-related quality of life in PLD?
- Is UDCA safe for PLD patients?
- What is the effect of UDCA on liver cyst volume in PLD?
- Does UDCA have a different effect on TLV in ADPKD and ADPLD patients?

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Part I

Who should we treat and what should we target?

Chapter 2

Risk factors for progressive polycystic liver disease: where do we stand?

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Nephrol Dial Transplant. 2016 Jun;31(6):857-9.

In the issue of *Nephrology Dialysis and Transplantation* Chebib *et al.* [1] analyze whether mutations in genes responsible for autosomal dominant polycystic kidney disease (ADPKD) contribute to its most frequent extrarenal phenotype: polycystic liver disease (PLD). In order to do so, they searched for mild (*PKD2*) and severe mutations (truncating and non-truncating *PKD1*) in a large cohort of ADPKD patients. They used liver imaging data to establish genotype-phenotype correlations. Though PKD genotype and renal phenotype are strongly related in ADPKD patients, this was clearly not the case for PKD genotype and PLD phenotype. These observations raise a number of questions. Is a cross- sectional analysis the best model to study genotype-phenotype associations? If PKD genotypes are not, which are bona fide risk factors for progressive liver enlargement in ADPKD? What future steps are required to elucidate determinants for liver cyst growth in ADPKD patients?

Polycystic liver disease

PLD is characterized by the presence of cysts throughout the liver and caused by mutations in specific genes such as *PKD1*, *PKD2*, *PRKCSH*, *Sec63* and *LRP5*. [2,3] Two genetically distinct disorders are associated with PLD, ADPKD and autosomal dominant polycystic liver disease (ADPLD). In ADPLD patients, PLD is the primary phenotype, while kidney cysts are ubiquitous in ADPKD. [4,5] Hepatic cysts are the most common extra-renal manifestation in ADPKD with a prevalence of 69% among women and 79% among men, and prevalence increases with age. [6] There is a wide range in liver size and liver growth rate among patients, but also between members from the same family who share identical genetic mutations. [2,7] In a small subset of patients, PLD leads to symptoms such as abdominal pain and early satiety, reduced health-related quality of life (HRQL) and complications such as infections or cyst rupture. [4,8] Reduction in liver volume is thought to improve HRQL and symptoms, although the relation between both elements remains unclear. Indeed, a study in 92 PLD patients did not establish a correlation between liver volume and physical component score of a generic short-form health survey. [8]

Genotype

ADPKD is a caused by mutations in *PKD1* and *PKD2*. Both genes are responsible for almost all ADPKD cases. *PKD1* and *PKD2* encode two transmembrane proteins, polycystin-1 and polycystin-2, that represent a subfamily of transient receptor potential channels and are located at the primary cilium. We have witnessed considerable progress towards our understanding of mechanisms that are involved in cyst growth in ADPKD. What had been established was that *PKD1* or *PKD2* germline mutations alone are insufficient for cystogenesis. Molecular studies of cyst epithelia found that the second affected ADPKD allele in cystic cells is often lost or mutated. In addition, clinical observations are consistent with a high intrafamilial phenotypic variation in related individuals with identical mutations. Indeed, somatic hit mutations in *PKD1* or *PKD2* have been identified in liver and kidney tissues of ADPKD patients, supportive of a second-hit model. [9-12]

Studies in ADPLD are in line with this concept. In ADPLD cyst tissue, loss of heterozygosity of the *PRKCSH* and *Sec63* allele is present with a frequency that appears to depend on the gene affected in germline. [9,10] There is no evidence that the gene (*PRKCSH*, *LRP5* or *Sec63*) or type of genetic mutation affects ADPLD phenotype. [13] One could hypothesize that patients with a severe phenotype are more susceptible to acquire somatic mutations than patients with a mild phenotype, or that patients with mild phenotypes have protective mechanisms that render the "resistance" against somatic mutations. The (unaddressed) issue central to this theme is, which factors drive somatic mutations?

In the current study, stratification of ADPKD patients according to PKD genotypes suggests that a clear relation between PKD genotype and PLD phenotype is absent. The question is whether a retrospective cross-sectional cohort study is the best design to assess genotype-phenotype associations. The authors limited their selection to patients with known *PKD1*/*PKD2* genotype, potentially introducing selection bias. Molecular genetic testing is not part of routine clinical practice but may be considered in cases with equivocal or atypical renal imaging findings (e.g. markedly asymmetric PKD, renal failure without significant kidney enlargement) or in ADPKD patients without a positive family history. [14] In addition, by including families, their dataset was enriched with patients who had similar genotypes, limiting the spread of the mutational spectrum and possibility to detect meaningful associations. From a phenotype perspective, patients were included if MRI/CT images were available. As per guidelines routine hepatic imaging is not recommended for follow-up of ADPKD patients; this may have led to the selection of a study population at the extreme ends of severity with respect to renal and/or hepatic phenotype. [14] Again, this would make it challenging to detect meaningful associations. The overall majority had *PKD1* mutations, which certainly limits the chance of detecting associations with *PKD2*.

It is likely that several disrupted gene products are involved in the considerable phenotype variability in PLD patients. The presence of modifier genes or epigenetic factors might contribute to this. [15] Modifier genes are able to modulate cystogenesis by determining the quality or quantity of second hits. The evidence for loss-of-heterozygosity regions in ADPLD cyst tissue might indicate that modifier genes affect cystogenesis in PLD. [7] In ADPKD patients, candidate gene or genome-wide association studies have not yet identified modifier genes for ADPKD phenotype. Larger international replication studies are needed to confirm these results. [15]

Another concept that might explain differences in phenotype expression in PLD is epigenetic modulation, a phenomenon that changes gene expression and activity without modifying DNA sequence. [16] Only recently evidence surfaced that epigenetics might be associated with renal pathogenesis in ADPKD. In a cohort of ADPKD and non-ADPKD individuals, genome-wide profiling showed that hypermethylation of *PKD1* and other genes involved in ion transport and cell adhesion in ADPKD patients resulted in downregulated expression and cystogenesis. These data suggest that epigenetic silencing might play a key role renal cyst development. [17,18] This

has never been studied in PLD, and further research needs to elucidate whether epigenetics have a main role in the complex of pathways involved in genotype-to-phenotype expression.

Patient factors

Patient factors that influence progression of PLD are age, female gender, oestrogen use and pregnancies. [13,19,20] There is an age-dependent effect, and the prevalence of hepatic cysts in a large cohort (*n* = 230) of early ADPKD rose from 58% in patients 15-24 years of age to 94% in 35-46 years old patients. [20] Clinical trials that included placebo groups have illustrated that particularly young women (≤48 years) demonstrate progressive cyst growth (4.8% in 6-12 months), compared with stagnant liver growth in women older than 48 years (0.6% in 6-12 months), and men (-0.1% in 6-12 months). [21] In this issue of *Nephrology Dialysis Transplantation*, Chebib *et al.* [1] confirmed these results; age younger than 48 yrs was associated with an annual growth rate of 2.65%, compared with 0.09% in women ≥48 years (P<0.001). In contrast, this age effect was not seen in men (2.28% vs. 0.8%, P=0.18). The concept that oestrogens are positive modulators of cholangiocyte proliferation is supported by a clinical trial that treated postmenopausal women with exogenous oestrogen. Liver volume significantly increased under oestrogen treatment (7±12% vs. 2±8%, P=0.03). [19] Additionally, a history of pregnancies is a strong predictor of presence of hepatic cysts. [19,22] A retrospective cohort study in 275 female PLD patients showed no effect of pregnancy or exogenous oestrogen on liver volume, although this difference in results might be explained by lower oestrogen content of contemporary contraceptives compared with the earlier days. Chebib *et al.* [1] also found no relation between pregnancy and liver volume. [6] In clinical practice, we still remain cautious in prescribing oral contraceptives or postmenopausal oestrogen to women with PLD. In our clinical practice, we advice patients to seek alternative contraceptives such as an intrauterine device, and explain the possible influence of pregnancies on liver volume.

Conclusion

Specific genes are associated with development of PLD in ADPKD and ADPLD patients. Currently, there is no evidence for a role of genotype in the phenotypic expression of PLD. The present study highlights the importance of assessing effect of genotype on phenotypic disease characteristics. It also demonstrates issues that come along with retrospective cohort studies. Patient-related factors such as age, sex, oestrogen use and pregnancies influence natural course (Figure 1). Nevertheless, it is still impossible to predict natural course in individual patients that impedes treatment of PLD. Who needs therapy and when is the best time to initiate therapy? These are the main questions for physicians who treat PLD patients. To answer these questions, we need to understand the factors that are involved in the phenotypic variability of PLD. Assuming the two-hit concept to be true, a model should be created that reveals the relation between factors that manipulate somatic mutations and eventually lead to different phenotypes. Epigenetic alterations and modifier genes might be key modulators.

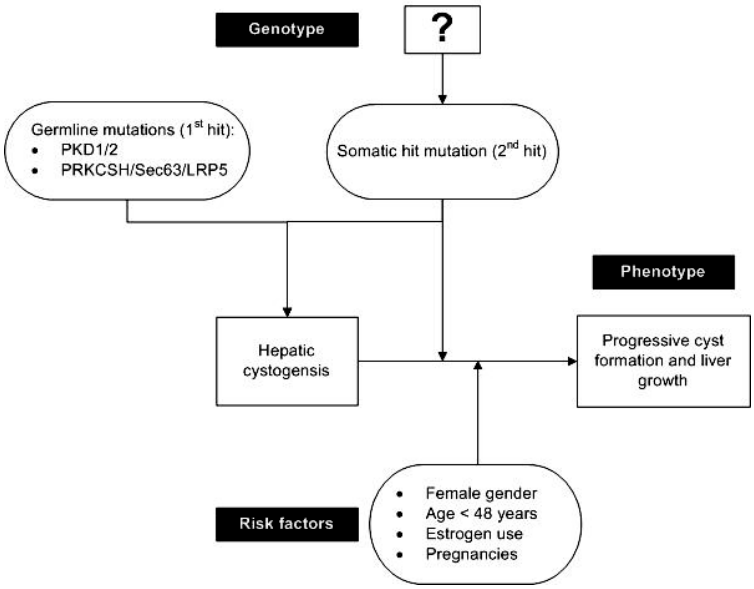

Figure 1. Genetic and patient factors involved in PLD.

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Chapter 3

The association of combined total kidney and liver volume with pain and gastrointestinal symptoms in patients with later stage ADPKD

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ABSTRACT

Background & aims

There is an ongoing debate if and how kidney and liver volume are associated with pain and gastrointestinal symptoms in ADPKD patients. Since both kidney and liver volume could interact, we investigated whether combined total kidney and liver volume had stronger associations with ADPKD-related pain and gastrointestinal (GI) symptoms than the volumes of the organs separately.

Methods

We used baseline data from the DIPAK-1 study which included ADPKD patients with an eGFR between 30-60 mL/min/1.73m^2. MR imaging was performed to measure height adjusted total kidney volume (hTKV), total liver volume (hTLV) and the combination of both (hTKLV).

Results

309 ADPKD patients were included with a mean age of 48±7 years, 53% female, eGFR 50±11 mL/min/1.73m^2 and median hTKV, hTLV and hTKLV of 1095 [758-1669], 1173 [994-1523] and 2496 [1972-3352] mL/m, respectively. ADPKD-related pain and GI symptoms were present in respectively 27.5% and 61.2% of patients. Sex was no effect modifier in the association between kidney and/or liver volume, and symptom burden. hTKLV and hTLV were significantly associated with pain and GI symptoms, whereas hTKV was not. Model testing revealed that the associations of pain and GI symptoms with hTKLV were significantly stronger than with hTKV (p=0.04 and p=0.04, respectively), but not when compared to hTLV (p=0.2 and p=0.5, respectively).

Conclusions

This study indicates that combined kidney and liver volume was associated with the presence and severity of pain and GI symptoms in ADPKD, with a more prominent role for hTLV than for hTKV.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive renal cyst formation and the majority of patients also have liver cysts (>94%) [1]. During lifetime kidney and liver volume increase, leading to distension of the renal and hepatic capsules, and compression of adjacent organs [2]. Consequently, a substantial proportion of ADPKD patients suffers from pain and gastrointestinal symptoms, such as abdominal fullness and early satiety[3-6]. There is an ongoing debate if and how kidney and liver volume are associated with pain and gastrointestinal symptoms. A number of studies have investigated symptom burden in ADPKD patients [5,7-9]. The largest of these studies did not find an association between kidney volume and pain, except in a small subgroup with very large kidneys [5]. Another study concluded that quality of life was not different between patients with a total kidney volume (TKV) larger or smaller than 1000 mL, but the effect of liver volume was not assessed [8]. Two studies that analyzed the effect of liver volume on quality of life, showed conflicting results, with one study finding no relation and the other a significant, but weak association between liver volume and symptom burden [10,11]. Of note, all aforementioned studies varied in the use of height or non-height adjusted kidney and liver volumes [5,7-10]. In terms of disease progression height adjusted total kidney volume (hTKV) has been shown to be more closely related to the rate of disease progression than non-height adjusted TKV [12]. The question arises whether the conflicting data in literature may be explained by the fact that sometimes height and sometimes non-height adjusted volumes were used to test correlations with symptom burden.

Another factor that potentially affects symptom burden is a difference in sex. In literature females are overrepresented among cohorts of patients with symptomatic ADPKD [13,14]. This is usually attributed to the presence of a more severe liver phenotype in females [15]. On the other hand, pain sensitivity has been suggested to be greater among females, and females are more likely to report gastrointestinal symptoms when compared to males [16-18]. To our knowledge, it has not been investigated whether higher symptom burden in females with ADPKD is caused by differences in reporting by sex in general, or by differences in kidney and/or liver size between both sexes.

Since both kidney and liver volume drive intra-abdominal volume, it is reasonable to assess the association of combined kidney and liver volume with ADPKD-related pain and gastrointestinal symptoms [19]. Therefore, we investigated in a large cohort of ADPKD patients whether combined kidney and liver volume is more strongly associated with ADPKD-related pain and gastrointestinal symptoms than kidney or liver volume alone, secondly whether there is a differences in the strength of this association between males and females, and thirdly whether height adjusted volumes are more strongly associated with pain and gastrointestinal symptoms than non-height adjusted volumes.

MATERIALS AND METHODS

Patients and study design

Baseline data were used from the DIPAK-1 study, an investigator driven, multi-center, randomized, controlled clinical trial that included ADPKD patients with an estimated glomerular filtration rate (eGFR) between 30-60 mL/min/1.73m^2 and age 18-60 years. Patients were enrolled at 4 University Medical Centers in the Netherlands (Groningen, Leiden, Nijmegen and Rotterdam) between June 2012 and March 2015. ADPKD diagnosis was based on the modified Ravine criteria [20]. Exclusion criteria were among others, concomitant illnesses likely to confound the natural decline of renal function in ADPKD, for example diabetes mellitus. Details of the study protocol have been published elsewhere [21]. The Medical Ethics Committee of the University Medical Center Groningen approved the protocol of the DIPAK-1 study that was conducted in accordance with the International Conference of Harmonization Good Clinical Practice Guidelines and in adherence to the ethics principles that have their origin in the Declaration of Helsinki (METc2012/060). All patients gave written informed consent.

Data collection, measurements and definitions

Evaluations were performed in all patients at baseline including standardized interviews, physical examination, collection of blood samples and MR imaging. During the interviews information was gathered about demographics, medical history, pain and gastrointestinal symptoms. Renal pain was defined as pain or discomfort located in the flank, the lower back or abdomen. Liver pain was defined as pain or discomfort located in the right upper abdomen, behind or below the rib cage. The severity of renal and/or liver pain during the last 4 weeks was assessed on a 1-10 scale (1=no pain, 10=worst possible pain), and presence of renal or liver pain was defined as a score >2. Since it is difficult to distinguish between renal and liver pain, we used a composite score for ADPKD-related pain. Presence of ADPKD-related pain was defined as a composite score of >2 on either renal or liver pain. For severity of ADPKD-related pain the highest score on either renal or liver pain was used. The presence of gastrointestinal symptoms over the last 4 weeks was recorded via the gastrointestinal symptoms questionnaire [22] (Supplementary file 1). This questionnaire contains 11 items including: lower and upper abdominal pain, heartburn, regurgitation, nausea, vomiting, loss of appetite, early satiety, dyspnea, increase of abdominal waist and involuntary weight loss. All symptoms were assessed using a 7-point Likert scale, ranging from 1 ("none") to 7 ("severe"). Symptom severity sum score was calculated by summing all scores and converting it to a score from 0 to 100 [22]. Presence of gastrointestinal symptoms was defined as a score of >2 on at least one of 11 gastrointestinal symptoms.

Serum creatinine was reported and used to estimate GFR (applying the CKD-EPI equation) [23]. All patients underwent a MRI to assess kidney and liver volumes by the manually tracing method using the commercially available software Analyze Direct 11.0 (Analyze Direct, Inc., Overland Park, KS, USA). Kidney and liver volumes were calculated from the set of contiguous images

by summing the products of the area measurements within the kidney or liver boundaries and slice thickness. Details of the imaging protocol have been reported previously [21]. hTKV, height adjusted total liver volume (hTLV) and combined total kidney liver volume (hTKLV) were calculated as total organ volume in mL divided by height in meters.

Statistical analyses

We performed a cross-sectional analysis of the baseline data of the DIPAK-1 study. Baseline characteristics were calculated for the overall population and stratified for patients experiencing ADPKD-related pain, experiencing gastrointestinal symptoms and sex. Parametric variables are expressed as mean ± standard deviation (SD), non-parametric variables as median ± interquartile range [IQR]. Differences in baseline characteristics between groups were calculated with a Chi-square test for categorical data, and for continuous data with Student's t-test or a Mann-Whitney U test in case of non-parametric data.

To investigate whether organ volume correlated with ADPKD-related pain and gastrointestinal symptoms, univariate and multivariate linear regression analyses were performed. hTKV, hTLV and hTKLV were logarithmic transformed to fulfill the requirement of normal distribution of the residuals for regression analysis. The multivariate linear analyses were subsequently adjusted for age and eGFR to correct for disease severity. To investigate differences between males and females the variable sex was added to the regression analysis. To explore whether associations between organ volume (i.e. hTKV, hTLV and hTKLV) and symptom burden (i.e. ADPKD-related pain and gastrointestinal symptoms) were different between males and females, interaction was tested by adding product terms (sex times volume) as independent variable to the models. We used bootstrapping (2000 times) to investigate whether the association of hTKLV with ADPKD-related pain and gastrointestinal symptoms was stronger than the associations between either hTKV or hTLV, and ADPKD-related pain and gastrointestinal symptoms. In all models we corrected for disease severity by adjustment for sex, age and eGFR. As sensitivity analysis, we restricted the analysis of the associations between organ volume and symptom burden to patients with extremely enlarged kidney volumes (hTKV >1000 mL/m), as defined previously in literature [5]. Lastly, bootstrapping was performed to analyze whether height adjusted volume models were more strongly associated with pain and gastrointestinal symptoms than non-height adjusted volume models. All analyses were performed using SPSS (software version 22.0, Chicago, IL, USA) and STATA (Version 14 StataCorp SE) statistical software, and a two-sided $p<0.05$ was considered to indicate statistical significance.

RESULTS

Patient characteristics

We enrolled 309 ADPKD patients in our study, of which 53% were female with a mean age of 48±7 years. Following our inclusion criteria all patients had an impaired renal function, with a mean eGFR of 50±11 mL/min/1.73m^2. Blood pressure was on average well controlled and almost all patients used antihypertensive medication (91.2%). Median height adjusted total kidney volume (hTKV), total liver volume (hTLV) and combined total kidney liver volume (hTKLV) were respectively 1095 [758-1669] mL/m, 1173 [994-1523] mL/m and 2496 [1972-3352] mL/m. Liver cysts were present in the large majority of patients (93.2%).

ADPKD-related pain and gastrointestinal symptoms

ADPKD-related pain was reported by 27.5% of the study population (renal pain: 24.9% and liver pain: 11.3%) (Table 1). Pain was more common in females than in males. Age and eGFR did not differ between patients with and without pain, while a history of renal pain, liver pain, urinary tract infection, renal cyst infection, liver cyst infection and macroscopic hematuria were more common in those who reported pain. Liver cysts were also more common in patients experiencing ADPKD-related pain. Larger hTLV and hTKLV were associated with pain, whereas hTKV was not.

A total of 61.2% of the ADPKD patients experienced gastrointestinal symptoms, with females being overrepresented in patients reporting these symptoms (Table 1). Age and eGFR were not different between patients with or without gastrointestinal symptoms. Presence of gastrointestinal symptoms was associated with a history of renal pain, liver pain, urinary tract infection, renal cyst infection and renal surgery. Out of the 11 gastrointestinal symptoms that were assessed, the most frequently reported symptom was early satiety (32.0%), followed by increased abdominal volume (25.2%), dyspnea (24.6%), heartburn (22.7%) and regurgitation (18.4%) (Table 2).

Association of kidney and liver volume with pain and gastrointestinal symptoms

To investigate whether associations between volumes (hTKV, hTLV and hTKLV) and symptom burden (ADPKD-related pain and gastrointestinal symptoms) were sex dependent, we tested the interaction between these characteristics. No significant interaction with sex was found, indicating that all associations could be tested across the complete study population and that stratification by sex was not necessary. hTKV was not associated with severity of ADPKD-related pain in the overall population (R=0.05, p=0.44) (Figure 1). In contrast, hTLV and hTKLV were both correlated with ADPKD-related pain (R=0.20, p<0.001 and R=0.23, p<0.001). After adjustment for disease severity, by correction for age, sex and eGFR, these associations remained significant (R=0.23, p<0.001 and R=0.20, p<0.001, respectively). The hTKLV model was also more strongly associated with pain than the hTKV model (p=0.04), whereas this was not the case for the hTLV model (p=0.2).

Table 1. Baseline characteristics of DIPAK study participants stratified according to presence or absence of ADPKD-related pain and gastrointestinal symptoms.

	Presence of ADPKD-related pain			Presence of gastrointestinal symptoms		
	Yes	**No**	**P-val.**	**Yes**	**No**	**P-val.**
N	85 (27.5)	224 (72.5)	-	189 (61.2)	117 (37.9)	-
Female sex (%)	56 (65.9)	106 (48.8)	0.006	111 (58.7)	52 (44.4)	0.02
Age (yrs)	48±7	48±7	0.6	48±8	48±7	1.0
Height (m)	1.75±0.1	1.77±0.1	0.05	1.75±0.1	1.79±0.1	0.001
Weight (kg)	82±16	85±17	0.3	84±18	85±15	0.6
BMI (kg/m^2)	26.9±4.4	27.0±4.8	1.0	27.2±4.7	26.5±4.5	0.2
History of						
Renal pain (%)	70 (82.4)	75 (34.2)	<0.001	105 (55.6)	40 (34.2)	<0.001
Liver pain (%)	27 (31.8)	10 (4.6)	<0.001	35 (18.5)	2 (1.7)	<0.001
UTI (%)	52 (61.2)	93 (42.5)	0.003	100 (52.9)	46 (39.3)	0.02
Renal cyst infection (%)	14 (16.5)	14 (6.4)	0.006	23 (12.1)	5 (4.2)	0.02
Liver cyst infection (%)	2 (2.4)	0 (-)	0.02	2 (1.1)	0 (-)	0.3
Macroscopic hematuria (%)	40 (47.1)	60 (26.9)	0.001	64 (33.9)	36 (30.8)	0.6
Renal surgery >1 year (%)	1 (1.2)	2 (0.9)	0.8	0 (-)	3 (2.6)	0.03
Liver surgery >1 year (%)	3 (3.5)	1 (0.5)	0.04	3 (1.6)	1 (0.9)	0.6
SBP (mmHg)	134±13	132±14	0.4	133±14	131±13	0.3
DBP (mmHg)	85±10	81±10	0.01	82±9	82±10	0.5
Use of BPLD (%)	82 (96.5)	195 (89.4)	0.05	173 (92.0)	105 (89.7)	0.5
Presence of hypertension (%)	80 (94.1)	189 (86.3)	0.1	169 (89.4)	102 (87.2)	0.6
Presence of liver cysts (%)	84 (100)	199 (92.6)	0.01	180 (97.3)	105 (90.5)	0.01
eGFR (mL/min/1.73m^2)	49±11	50±11	0.4	49±11	50±10	0.5
TKV (mL)	2054 [1423-3319]	1910 [1256-2868]	0.4	2119 [1380-3185]	1809 [1246-2668]	0.05
hTKV (mL/m)	1193 [809-1869]	1056 [719-1646]	0.3	1221 [784-1796]	982 [684-1489]	0.02

Table 1. Continuation

	Presence of ADPKD-related pain			Presence of gastrointestinal symptoms		
	Yes	**No**	**P-val.**	**Yes**	**No**	**P-val.**
TLV (mL)	2300 [1908-4334]	2031 [1744-2556]	0.001	2148 [1803-3075]	2010 [1717-2474]	0.02
hTLV (mL/m)	1345 [1080-2435]	1149 [986-1418]	<0.001	1219 [1023-1699]	1144 [955-1367]	0.003
TKLV (mL)	5366 [3954-6955]	4182 [3402-5500]	<0.001	4645 [3698-6491]	4002 [32925091]	<0.001
hTKLV (mL/m)	2979 [2186-3921]	2392 [1931-3030]	<0.001	2661 [2135-3617]	2216 [1873-2854]	<0.001

Abbreviations are: BMI, body mass index; UTI, urinary tract infection; SBP, systolic blood pressure; DBP, diastolic blood pressure; BPLD, blood pressure lowering drug; eGFR, estimated glomerular filtration rate; TKV, total kidney volume; hTKV, height adjusted total kidney volume; TLV, total liver volume; hTLV, height adjusted total liver volume; TKLV, total kidney liver volume; hTKLV, height adjusted total kidney liver volume.

Data are shown as number (%), mean± standard deviation or median [interquartile range

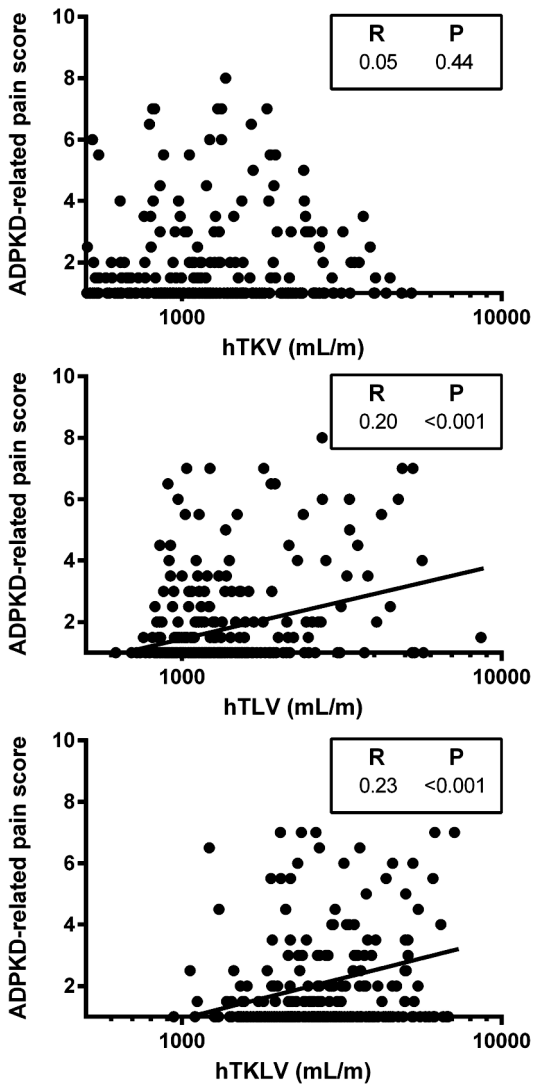

Figure 1. Associations of height adjusted Total Kidney Volume (hTKV), Total Liver Volume (hTLV) and combined Total Kidney Liver Volume (hTKLV) with ADPKD-related Pain Score (1-10).

We then tested whether kidney and liver volume were associated with gastrointestinal sum score. No association was found for hTKV (R=0.10, p=0.09), whereas hTLV and hTKLV were both associated with the gastrointestinal sum score (R=0.23, p<0.001 and R=0.23, p<0.001, respectively) (Figure 2). Again, the association with gastrointestinal symptoms was significantly stronger for the model containing hTKLV compared with the model containing hTKV (p=0.04), but not compared with the model with hTLV (p=0.5).

Of note, we performed a sensitivity analysis to test whether these associations were different in patients with larger kidneys (hTKV >1000 mL/m). Essentially the same results were found as in the initial analysis; hTLV and hTKLV were, and hTKV was not associated with ADPKD-related pain and gastrointestinal symptoms.

Differences in symptom burden between males and females

Renal and liver pain were present in 30.1% and 17.8% of females while this only accounted for 19.2% and 4.1% in males (p=0.04 and p<0.001, respectively). In case a patient experienced renal or liver pain, the severity of pain was similar among males and females. Gastrointestinal symptoms were more prevalent among females. The following symptoms were reported more frequently by females: abdominal pain, nausea, early satiety and an increased abdominal volume, compared to males (Table 2). Gastrointestinal symptoms as expressed in the gastrointestinal sum score were more severe in females than in males (17.6 vs. 9.0, p<0.001).

Females had larger hTLV and smaller hTKV than males (hTLV: 1249 [1034-1901] vs. 1130 [967-1336] mL/m, p<0.001 and hTKV: 923 [604-1330] vs. 1314 [935-2145] mL/m, p<0.001). hTKLV did not differ between both sexes (females: 2424 [1939-3213] mL/m, males 2537 [2065-3547] mL/m, p=0.2). Female sex was positively associated with symptom burden in ADPKD patients, but after adjustment for hTLV, this association lost significance.

Height adjusted versus non-height adjusted models

No difference was observed in the association with symptoms between the models with either hTKV or TKV (p=1.0), whereas the models with hTLV and hTKLV had stronger associations with pain than the models with TLV and TKLV (p=0.02 and p=0.01, respectively). For gastrointestinal sum score, similar results were found. hTLV and hTKLV models were more strongly associated with gastrointestinal symptoms than non-height adjusted models (p=0.01 and p=0.01, respectively), which did not account for the hTKV model (p=1.0). Of note, the results of correlation analyses of ADPKD-related pain and gastrointestinal symptoms with non-height adjusted TKV, TLV and TKLV, were essentially similar to the results of the primary analyses with hTKV, hTLV and hTKLV (Table 3 and Supplementary Table 1). The relations between organ volume and symptom burden still existed, but were less strong compared to the height adjusted models (Table 3).

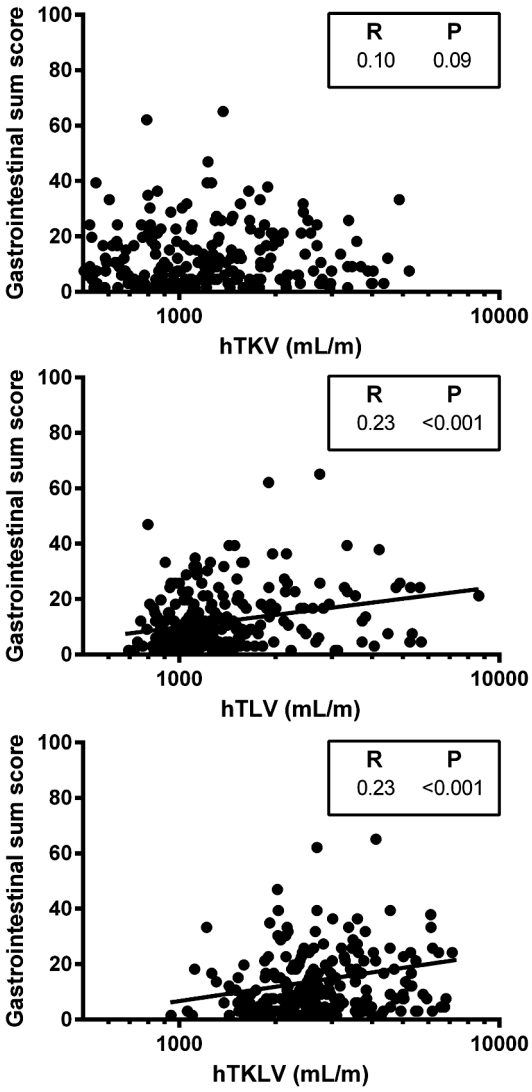

Figure 2. Associations of height adjusted Total Kidney Volume (hTKV), Total Liver Volume (hTLV) and combined Total Kidney Liver Volume (hTKLV) with gastrointestinal sum score (0-100).

Table 2. Prevalence and severity of ADPKD-related pain and gastrointestinal symptoms overall and stratified for sex.

	Overall % or median ± IQR	Males % or median ± IQR	Females % or median ± IQR	P-val.
History of pain				
Renal related pain	47.6%	43.2%	51.5%	0.14
Liver related pain	12.0%	2.1%	20.9%	<0.001
Renal or liver related pain	50.8%	45.2%	55.8%	0.06
Presence of pain				
Renal related pain	24.9%	19.2%	30.1%	0.04
Liver related pain	11.3%	4.1%	17.8%	<0.001
Renal or liver related pain	27.5%	19.9%	34.4%	0.006
Severity of present pain				
Renal related pain	4 [3-6]	3.5 [3.0-6.0]	5.0 [3.0-6.0]	0.3
Liver related pain	5 [4-7]	4 [3.8-4.8]	6 [4-7]	0.1
Renal or liver related pain	4 [3-7]	4.0 [3.0-6.0]	5.0 [3.0-7.0]	0.2
Gastrointestinal symptoms				
Lower abdominal pain	14.9%	9.6%	19.6%	0.02
Upper abdominal pain	17.8%	9.6%	25.2%	<0.001
Heartburn	22.7%	22.6%	22.7%	0.9
Regurgitation	18.4%	17.8%	19.0%	0.9
Nausea	13.6%	6.8%	19.6%	0.001
Vomiting	3.2%	2.1%	4.3%	0.3
Loss of appetite	16.2%	10.3%	21.5%	0.01
Early satiety	32.0%	19.9%	42.9%	<0.001
Dyspnea	24.6%	19.2%	29.4%	0.05
Increasing abdominal volume	25.2%	16.4%	33.1%	0.001
Involuntary weight loss	2.9%	1.4%	4.3%	0.1
Severity of present GI symptoms				
GI- sum score	12.0 [8.0-21.0]	9.0 [4.5-16.7]	17.6 [15.2-23.1]	<0.001

Abbreviations are: GI, gastrointestinal. Denominators depend on the number of patients who provided an answer for a specific question in the questionnaire. Renal and liver pain measured on scale 1-10 (1= no pain); GI-sum score ranging from 0-100. (0 = no symptoms).

Table 3. Associations of height adjusted kidney and liver volumes with pain and gastrointestinal symptoms.

	hTKV		hTLV		hTKLV	
	R	**P-val.**	**R**	**P-val.**	**R**	**P-val.**
History of pain						
Renal related pain	0.10	0.1	0.15	0.01	0.22	<0.001
Liver related pain	-0.06	0.3	0.30	<0.001	0.20	0.001
Renal or liver related pain	0.12	0.1	0.21	<0.001	0.27	<0.001
Presence of pain						
Renal related pain	0.07	0.2	0.16	0.01	0.21	<0.001
Liver related pain	0.01	0.8	0.25	<0.001	0.21	<0.001
Renal or liver related pain	0.06	0.3	0.21	<0.001	0.24	<0.001
Severity of present pain						
Renal related pain	0.04	0.5	0.14	0.02	0.17	0.003
Liver related pain	0.04	0.5	0.27	<0.001	0.26	<0.001
Renal or liver related pain	0.02	0.8	0.20	<0.001	0.22	<0.001
Gastrointestinal symptoms						
Lower abdominal pain	0.06	0.3	0.10	0.1	0.09	0.1
Upper abdominal pain	0.03	0.6	0.22	<0.001	0.19	0.001
Heartburn	0.15	0.01	0.05	0.4	0.13	0.03
Regurgitation	0.12	0.03	0.14	0.02	0.15	0.01
Nausea	-0.04	0.5	0.22	<0.001	0.11	0.05
Vomiting	-0.02	0.8	0.13	0.03	0.09	0.1
Loss of appetite	0.01	0.8	0.18	0.002	0.16	0.01
Early satiety	0.06	0.3	0.21	<0.001	0.21	<0.001
Dyspnea	0.06	0.3	0.14	0.02	0.11	0.1
Increasing abdominal volume	0.12	0.03	0.15	0.01	0.22	<0.001
Involuntary weight loss	-0.02	0.7	0.08	0.2	0.00	1.0
Severity present gastrointestinal symptoms						
GI- sum score	0.10	0.1	0.23	<0.001	0.23	<0.001

Abbreviations are: hTKV, height adjusted total kidney volume; hTLV, height adjusted total liver volume; hTKLV, height adjusted total kidney liver volume; GI, gastrointestinal. hTKV, hTLV and hTKLV were log transformed. Denominators depend on the number of patients who provided an answer for a specific question in the questionnaire. Renal and liver pain measured on scale 1-10 (1= no pain); GI-sum score ranging from 0-100. (0 = no symptoms).

DISCUSSION

This study showed that both hTKLV and hTLV were moderately associated with pain and gastrointestinal symptoms in patients with later stage ADPKD, while hTKV was not. Other patient related characteristics, such as a history of urinary tract infection, renal cyst infection, liver cyst infection and macroscopic hematuria, were also associated with symptom burden. We found that females more frequently suffered from symptoms than males. However, sex was not an effect modifier in the relation between organ volume and symptoms and the higher symptom burden in women seems to be explained by their larger hTLV. In addition, the models containing height adjusted organ volumes were more strongly associated with pain and gastrointestinal symptoms compared to non-height adjusted models.

The general assumption is that a large kidney volume in ADPKD plays a role in causing pain [2]. Interestingly, two studies that investigated the association between kidney volume and pain, did not confirm this assumption [5,8]. The authors found that total kidney volume did not differ between those patients taking or not taking analgesics[8]. Only at the extreme of renal volumes in ADPKD (hTKV >1000 mL/m), an association between kidney volume and pain was found [5]. In our study no association was found between hTKV and pain in the overall study population, nor in patients with very large kidneys. The present data add therefore to the evidence that the link between hTKV and pain is weak or even absent.

Previous studies found inconsistent results regarding the relation between liver volume and symptom burden. One study by Hogan et al, that included patients with early stage ADPKD (eGFR >60 mL/min/1.73m^2), found an association between liver volume and reduced quality of life [10]. However, another study found no such relation in 92 patients with polycystic liver disease, of whom 67% had ADPKD [11]. Of note, this latter study included only patients with symptomatic polycystic liver disease, which makes finding associations between symptoms and liver volume population difficult. Our results suggest, in accordance with the results of Hogan et al, that liver volume in ADPKD contributes significantly to symptom burden, as both hTLV and hTKLV were associated with pain and gastrointestinal symptoms. The reason why liver volume seems to play a more important role in causing symptoms than kidney volume cannot be concluded from the present data. However, we hypothesize that organ location might be important. The liver has a position more closely to other intra-abdominal organs than the kidneys, that are located retroperitoneal. An increase in liver volume may consequently lead to more compression of adjacent tissues (i.e. stomach, intestines and lungs) than an increase in kidney volume, causing symptoms such as dyspepsia, early satiety, dyspnea and pain [4].

Only one previous study has investigated the role of combined total kidney liver volume on patient reported outcome measures and found no association with health related quality of life [7]. Of note, kidney and liver volumes were available in only 31 out of 219 included patients (of which 21 were on dialysis) and the lack of significant associations may be due to the small

sample size. In contrast, we found significant association between hTKLV, hTLV and symptoms. It should be noted, however, that the strength of these associations was moderate. This suggests that symptom burden is multifactorial and that other factors may contribute [7]. Potential other determinants may include coping mechanisms and comorbidity, such as a history of urinary tract infection, renal cyst infection, liver cyst infection and macroscopic hematuria, which according to our results, were also related to current ADPKD-related symptom burden. Adequate management of these events may be indicated to reduce the presence of symptom burden in ADPKD.

Our data indicate a gender disbalance in prevalence and severity of ADPKD-related pain and gastrointestinal symptoms. This is in accordance with earlier studies that found that females more frequently reported pain, used analgesics and were more impaired in their physical activities compared to males [5]. The same observation is true for the general population, where females report pain and gastrointestinal symptoms more frequently [16-18]. Surprisingly, sex was no effect modifier in the relation between volumes and symptom burden in our study. As expected, females had larger hTLV compared to males, and when adjusted for hTLV, variations in symptom burden between males and females disappeared. Based on these data we hypothesize that the higher symptom burden in women could be explained by their larger hTLV, though it might be that women experience more pain in general, compared to men. Despite these findings, physicians have to realize that symptomatic polycystic liver disease will mainly be present in females, as estrogens stimulate liver cyst growth [24,25]. Therefore the use of estrogens, such as in oral contraceptives, should be discouraged in symptomatic female ADPKD patients.

In symptomatic ADPKD patients, therapies are indicated that can slow cyst growth in both kidneys and liver. The TEMPO 3:4 trial demonstrated that tolvaptan, a vasopressin V2 receptor antagonist, decreased the rate of growth in total kidney volume[26]. This study also suggested that tolvaptan had a positive effect on acute renal pain events[26]. In contrast to the beneficial effect on renal cyst growth, tolvaptan presumably has no effect on liver cyst growth because the V2 receptor is not expressed in liver tissue. Our results suggest that in order to effectively reduce ADPKD-related symptom burden, therapy should also target liver cysts. Somatostatin analogues have been shown to reduce liver growth rate are and symptoms in ADPKD patients with severe polycystic liver disease [13,14,27]. These agents also hold promise to reduce the rate of growth of total kidney volume [13,28] and the rate of renal function decline in ADPKD patients [29]. Somatostatin analogue therapy may therefore become a treatment option in ADPKD patients who suffer from pain and gastrointestinal symptoms, but this issue needs additional study before somatostatin analogues can be prescribed in clinical practice. Two randomized controlled trials are ongoing to test the efficacy of somatostatin analogues to delay disease progression and reduce symptom burden in ADPKD [21,30].

A limitation of our study is that it is performed in the setting of a randomized controlled trial with specific inclusion criteria for age (18-60 years) and renal function (eGFR 30-60 mL/min/1.73m^2).This may make extrapolation of our findings to the general ADPKD population difficult. However, we observed that neither ADPKD-related pain, nor gastrointestinal symptoms were associated with renal function, suggesting that our results may be valid for the general ADPKD population. The main strength of our study is the systematic and prospective nature of data collection, that resulted in a well-phenotyped population.

In conclusion, we found that combined kidney and liver volume is associated with pain and gastrointestinal symptoms in ADPKD, with a more prominent role for liver volume than for kidney volume. It should be noted, however, that other determinants, such as a history of urinary tract infection, renal cyst infection, liver cyst infection and macroscopic hematuria, also seem to be of importance in determining symptom burden in ADPKD. Height adjusted organ volumes were more strongly associated with symptom burden compared to the non-height adjusted organ volumes, emphasizing the relevance of height adjustment to assess associations with symptom burden. Female ADPKD patients more often experienced pain and gastrointestinal symptoms than males. This sex difference could be explained by larger liver volumes in females compared to males. Lastly, our results implicate that physicians should be aware of the role of liver volume in symptomatic ADPKD and that efforts to reduce symptom burden should target especially liver volume.

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SUPPLEMENTARY FILES

Supplementary File 1. Gastrointestinal symptoms questionnaire

Did you experience during the ***last 4 weeks***	**None**	**Mild**	**Mode-rate**	**Quite a lot**	**Freq-uently**	**Severe**	**Very severe**
1. Lower abdominal pain	O	O	O	O	O	O	O
2. Upper abdominal pain	O	O	O	O	O	O	O
3. Heartburn	O	O	O	O	O	O	O
4. Regurgitation	O	O	O	O	O	O	O
5. Nausea	O	O	O	O	O	O	O
6. Vomiting	O	O	O	O	O	O	O
7. Loss of appetite	O	O	O	O	O	O	O
8. Early satiety	O	O	O	O	O	O	O
9. Shortness of breath	O	O	O	O	O	O	O
10. Increase of abdominal waist	O	O	O	O	O	O	O
11. Involuntary weight loss	O	O	O	O	O	O	O

Supplementary Table 1. Associations of kidney and liver volumes with pain and gastrointestinal symptoms (not height-adjusted).

	TKV		TLV		TKLV	
	R	**P-val.**	**R**	**P-val.**	**R**	**P-val.**
History of pain						
Renal related pain	0.10	0.2	0.16	0.006	0.21	<0.001
Liver related pain	-0.08	0.2	0.28	<0.001	0.16	0.006
Renal or liver related pain	0.11	0.1	0.20	<0.001	0.26	<0.001
Presence of pain						
Renal related pain	0.06	0.3	0.15	0.008	0.19	0.001
Liver related pain	0.00	1.0	0.23	<0.001	0.19	0.001
Renal or liver related pain	0.05	0.4	0.20	<0.001	0.22	<0.001
Severity of present pain						
Renal related pain	0.03	0.6	0.13	0.03	0.16	0.01
Liver related pain	0.02	0.8	0.25	<0.001	0.23	<0.001
Renal or liver related pain	0.02	0.8	0.19	0.001	0.20	0.001
Gastrointestinal symptoms						
Lower abdominal pain	0.04	0.5	0.07	0.2	0.07	0.2
Upper abdominal pain	0.02	0.8	0.20	0.001	0.17	0.004
Heartburn	0.1	0.01	0.05	0.4	0.12	0.03
Regurgitation	0.11	0.05	0.13	0.03	0.14	0.01
Nausea	-0.06	0.3	0.19	0.001	0.08	0.2
Vomiting	-0.02	0.7	0.12	0.04	0.07	0.2
Loss of appetite	0.00	1.0	0.16	0.006	0.13	0.02
Early satiety	0.04	0.5	0.18	0.002	0.17	0.003
Dyspnea	0.04	0.5	0.11	0.05	0.08	0.2
Increasing abdominal volume	0.10	0.1	0.12	0.03	0.20	0.001
Involuntary weight loss	-0.03	0.6	0.07	0.2	-0.01	0.9
Severity present gastrointestinal symptoms						
GI- sum score	0.07	0.2	0.20	0.001	0.20	0.001

Abbreviations are: TKV, total kidney volume; TLV, total liver volume; TKLV, total kidney liver volume; GI, gastrointestinal. TKV, TLV and TKLV were log transformed. Denominators depend on the number of patients who provided an answer for a specific question in the questionnaire. Renal and liver pain measured on scale 1-10 (1= no pain); GI-sum score ranging from 0-100. (0 = no symptoms).

Part II

Who do we treat?

Chapter 4

Creating an effective clinical registry for rare diseases

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ABSTRACT

The exposure of clinicians to patients with rare gastrointestinal diseases is limited. This hurts clinical studies, which impedes accumulation of scientific knowledge on the natural course, treatment outcomes and prognosis in these patients. An excellent method to detect patterns on an aggregate level that would not be possible to discover in individual cases, is a registry study. This paper aims to describe a template to create a successful international registry for rare diseases. We focus mainly on rare hepatic diseases, but lessons from this paper serve other fields in medicine, as well.

INTRODUCTION

Increasing our knowledge about rare liver disorders, commonly defined as a disorder that affects < 1 in 2000 citizens, is imperative. [1] Because most physicians are not exposed to large numbers of rare disease patients, their knowledge on the natural course, treatment response and prognosis for that rare disease is incomplete. These difficulties clearly limit our understanding and are an obstacle for research efforts to improve the outlook of patients with rare diseases.

Registries may be the answer to the lack of solid evidence. By definition, a registry is an organized system that uses observational study methods to collect existing or uniform clinical data from individual patients. [2] A registry offers a unique opportunity to conduct research on populations and conditions that are not generally studied in clinical trials, yet are important to clinical decision-makers. [3]

The steps in creating a registry study do not differ much from the implementation of a clinical trial. All the fundamental elements, such as design, study population, timeline and data management are likewise present. By contrast, there is no standard guidance as to how to design a registry. A helpful open access resource is *Registries for Evaluating Patient Outcomes: A User's Guide* [2], from the Agency for Healthcare Research and Quality.

The purpose of this article is to provide a methods-based paper on how to develop an effective clinical registry for rare hepatic disorders (Table 1). The most important aspects that are part of the decision process are discussed, in view of our own experiences, and highlighted by examples from successful rare liver disease registries in literature. As such, the lessons from our paper can be applied to other fields in medicine, as well.

Table 1. A practical guide to develop a clinical registry in 5 steps

1. Define your goal	• Define a clear goal: is a registry the right approach for this?
2. Create a network	• Identify and include stakeholders • Keep them updated regularly • Make the registry transparent
3. Write your protocol	• Define the target population by broad inclusion criteria • Identify a (small!) core dataset of most relevant data variables • If possible, include PROMs
4. Collect high quality data	• A web-based datamanagement system is advised as it allows decentralized (international) data entry and quality checks
5. Termination of data collection and disseminating results	• If a registries has a fixed or open end depends on the purpose • A feedback loop improves continuous commitment to the project and supports dissemination of results

Objectives

The most important task before initiating a registry study is to define the main goal. Dividing your main goal into specific objectives and outcome measures will help you to decide on the best registry design. Registry studies can be created to address a broad spectrum of questions. We will illustrate this by using several examples that demonstrate the impact of international multicenter databases on clinically relevant issues (Table 2).

Table 2. Examples of multi-country liver disease registries founded in Europe

Name	Founding country	Participating countries	Size (~)	Website
Hepatitis delta registry	Germany	11	UK	http://hepatitis-delta.org/
DILI registry	Spain	1	901 cases 864 patients	http://www.spanishdili.uma.es/index.php/es/
Spanish Latin American DILI Network	Spain	9	190 cases 181 patients	-
PLD registry	the Netherlands	4	> 500 patients	-
European liver transplant registry	France/ Germany/U.K.	27	106.849 patients 118.441 LTx	http://www.eltr.org/
International PSC Study Group	Norway	>17	7.312 patients	http://www.ipscsg.org/

Abbreviations: DILI, drug-induced liver disease, EU, European Union; LTx, liver transplantation; PLD, polycystic liver disease; PSC, primary sclerosing cholangitis; UK, unknown; U.K., United Kingdom

Natural course, quality of life and epidemiology

One of the goals of a registry could be to study the natural course of disease and associated factors. We designed a polycystic liver disease (PLD) registry with exactly this in mind. PLD is a disorder where patients progressively develop liver cysts. Information on the natural course of PLD, and answers to questions such as what are the predictors of an aggressive disease course are lacking, to date. This registry will help us to elucidate the behavioral risk factors for disease and assess differences in treatment choices between countries. [4,5]

The UK Primary Biliary Cirrhosis (UK-PBC) collaboration is an excellent example of a network that already established a large successful national registry. [6] Primary biliary cirrhosis (PBC) is a rare disease (with a prevalence of 30 per 100.000 individuals in the population) with a

highly variable phenotype and a high prevalence among women (the male to female ratio is 1:10). [7] The sheer size of this registry makes it possible to study the clinical profile seen in a subgroup of male PBC patients. In addition, this consortium recently developed a UK-PBC risk score, to assess prognosis in PBC patients. [6] Finally, this registry enables mapping of the natural history of the disease in the total PBC population, to link genetic susceptibility with phenotype and outcome, and to study the impact of PBC on the patients' quality of life. [7,8] On a different note, registry studies facilitate studies on incidence and prevalence. A requirement is that they sample cases from a confined geographical area. Studies from the Primary Sclerosing Cholangitis (PSC) Study Group are a fine example, where al PSC patients in an area of six adjacent provinces were identified, comprising 50% of the Dutch population. [9]

Long-term efficacy

In order to study the long-term efficacy of therapeutic interventions, a registry is a perfect tool. Indeed, the relative probability of death and graft loss after primary liver transplantation (LTx) for a number of rare liver disorders is difficult to estimate. This is the reason for the European Liver Transplant Registry, [10] which collects data on death and graft loss as rare outcome measures in 8.840 transplanted patients.

Safety

A patient registry can be used to investigate safety, by collecting data on the unexpected adverse events of drugs. Drug-induced liver injury (DILI) is the most cited reason why approved drugs are withdrawn from the market by the US Food and Drug Administration (FDA). [11] Bromfenac and troglitazone are two well-known examples of drugs that were withdrawn because of severe hepatotoxicity that became apparent in the post-approval period. [12] A specific registry, such as the Spanish DILI Registry, collects real-life data of drug safety; and therefore, allows better estimation of the magnitude of side effects of a drug, in terms of incidence or prevalence.

Cost-effectiveness

Registries are a tool to investigate cost-effectiveness. This has become an important aspect of the market access package for novel interventions. The National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) database has been used to measure comparative treatments and the cost-effectiveness of treatment modalities for hepatocellular carcinoma (HCC). This has resulted in a clear picture of the costs of treatment modalities (LTx, chemotherapy, radiation, resection or no treatment) over various HCC stages, in relation to survival (effectiveness). [13] It goes without saying that registries such as the SEER database can be used to address other related questions. [14]

MATERIAL AND METHODS

Study population

Target population.

The purpose of a registry is a key factor that determines the target population. This is the population for whom the results are relevant, but at the same time are the source of the registry data. The actual population is a mere reflection (and probably a fraction) of the complete patient population. Only in case of an extremely rare disease is it possible to reach a coverage rate that approaches completeness. For example, the Dutch national Multiple Endocrine Neoplasia Type 1 database has been able to capture >90% of the total patient population in The Netherlands. [15] This contrasts with the situation in PBC, as the UK-PBC group has managed to include approximately 25% of all PBC patients in the UK. [7]

In order to appreciate the variability in phenotypic presentation of a disorder such as PLD, it is paramount to sample a large number of patients who are followed for a considerable time period. We have found it difficult for PLD to have watertight disease definition. A cut-off of the number of cysts (as the presence of > 20 liver cysts) is rather arbitrary and is not always strictly used by physicians. Some PLD mutation carriers (who most likely will develop the disease phenotype, with time) do not have the required number of cysts and may be asymptomatic at the time of inclusion. The use of overly strict inclusion criteria enhances the risk of exclusion of relevant patient populations, which leads to sampling bias, compromising external validation of results. Therefore, it is key to consider the consequences of having too strict inclusion criteria. For some diseases, there is a wide variation in terms of disease complexity and the treatment strategies between university and general district hospitals. In view of this, the UK-PBC consortium managed to include thousands of patients from general centers, as well as specialist centers across the entire UK. [7] This resulted in a geographically representative cohort, avoiding specialist center bias. A large epidemiological study in PSC patients highlights the influence of selection and/or referral bias in population-based studies. The median survival until liver transplantation or PSC-related death was 13.2 years in tertiary referral centers, while transplant-free survival was 21.3 years in the total cohort (p<0.0001). This highlights that it is paramount to collect data from university and general district hospitals, as well as tertiary referral centers, for accurate assessment of survival in uncommon diseases such as PSC.[9]

Design

International collaboration.

National and international collaboration are crucial, in order to collect a large study population. Isolated PLD is a rare liver disease with a prevalence of 1 in 158.000 people, and may also occur in the context of autosomal dominant polycystic kidney disease, which carries a prevalence of 1 in 1000. [16,17]. Currently, our local registry includes approximately 500 patients. We used our professional network, established for clinical trials, in order to achieve a larger

study population. Promoting your registry online or by presentations supports visibility of the project, and enables collaboration with international researchers.

The global PBC Study Group is a multicenter collaboration between 15 centers that have developed a registry, including the medical information of almost 5000 PBC patients in Europe and North America, based on individual databases. [18] These data were used to develop a validated scoring system to predict transplant-free survival in ursodeoxycholic acid-treated PBC patients and to elucidate predictors for development of HCC. [19,20] International successes like these emphasize that combining several national databases constitutes a unique opportunity to obtain the power to execute studies.

International cohort studies facilitate our understanding of heterogeneity in rare diseases, by stratification of at-risk groups. Risk stratification helps to identify the patient subgroups with low and high risk profiles; and allows us to select the patients whom have the greatest potential to benefit from treatment. [21,22] The GLOBE-score is a validated risk stratification tool that predicts transplant-free survival of PBC patients whom were treated with ursodeoxycholic acid, leading to more stratified and evidence-based individualized care. [19]

Stakeholders.
In the process of creating a registry, it is pivotal to consider the target audience for whom the outcomes matter. The identification of stakeholders is key to help determine the objectives of a registry, as they have an essential role in using or disseminating results from a registry. Patients, physicians, scientific societies, insurance companies, hospital staff and policymakers who may have a vested interest in the development of the registry, should be involved; and they are needed for public support. Some key success factors are engagement, i.e. the active influence on registry-shaping and long-term commitment. This can be achieved by organizing open sessions with different stakeholders, to introduce the concept of a registry in an early phase of registry development. In addition, it is important to motivate all parties by making the benefits of the registry visible. For example, authorship is important for the visibility of individual participants; and it is advisable to set up agreements on authorship, early in the process of registry development.

Data management.
A reliable data management system is essential. Direct communication between electronic patient records and registries would be ideal for the collection of registry data, as it saves money and time. Since most hospital systems are not yet set up to accommodate this, the most accurate and reliable method to collect data is through the creation of a web-based data management system. Though costs are higher in comparison to a non-electronic data management system, it enhances quality; as validation rules can be formulated that allow monitoring of data integrity. The host of a web-based registry can determine which role the data collectors will have in the

electronic environment. Every role comes with its own responsibilities. There can be a role for patients, in order to complete a questionnaire, or for researchers who collect their medical data. Another benefit of a web-based registry is that it allows decentralized data entry; and thus, the possibility to collect data internationally. Examples of electronic international registries are the Hepatitis C virus-TARGET and the Hepatitis Delta International Network, both of which were used for longitudinal observational studies. [23,24] An electronic registry is a financial investment, but in view of quality monitoring and efficiency, it will certainly pay off.

Timeline.
Registries can have a fixed or open-end timeline, depending on the overall purpose of the registry. Most studies using a registry as observational method have begun as open-ended projects, without a pre-defined stopping point. If continuation of the registry does not add any valuable information to the already captured data, the registry should be terminated and its data reported. [2]

Data collection
Data elements.
Data collection is a time-consuming process; and it is essential to consider all data elements that are central to the objective of the registry, to avoid the collection of high volumes of data with limited value. What helps in this process is to divide the main goals into specific objectives, subdividing further into measurable outcomes. [2] For example, our goal is to study the natural and clinical course of PLD, so one important objective is to obtain information on the determinants associated with treatment. As such, we need to include at least the following elements: current age, gender, age at diagnosis, date of first treatment and treatment strategy. We used an expert panel in order to capture all relevant variables. Ultimately, a small number of the most important variables remained. [25]

Self-reported data by patients and patient-reported outcome measures.
Collection of variables in patient registries can be performed by patients, researchers or physicians depending on the origin of data. Another option is to involve patients in this process. The UK-PBC group has utilized this concept, as the authors used self-reporting information from a large national cohort of PBC patients (n = 2353). For items such as age at diagnosis and therapy for PBC, it is recommended to cross-check self-reported data with the medical record data; but their results showed a high correlation, suggesting a high level of accuracy of the self-reported data. [7]

As the patient's view on their health status and treatment preferences has obtained a central position in the choice of treatment strategies, it is desirable to include the patient-reported outcome measures (PROMs). PROMs are ideal instruments to measure health gain. [26,27] This development is endorsed, as illustrated by the guidance on PROMs that is offered by the US FDA. [28] Web-based questionnaires are an ideal modality to collect PROMs. [29]

Data quality

Data quality and monitoring.

All elements that are included in a registry should be pre-defined; so that during data collection, it is clear to the data collector which information should be entered. For our PLD registry, we tested whether all definitions were interpreted in the same manner, by performing a pilot study. Two researchers collected data from medical records from the same patients, and the results were compared. We were able to clarify obscurities and vague definitions, and include some missing questions or variables. In order to verify reliability and reproducibility of data, several options are possible. The gold standard for data entry is the double entry of 5-10% of all patient points, to check and verify. [30] An even better option is to include a quality and control committee, for central and/or local monitoring, in order to guarantee the quality of data. Such a committee should monitor electronic data collection and visit different sites for quality checks. By formulating validation rules in the electronic data management system, incorrect or inconsistent (for instance premenopausal status in men) data can be easily found and rectified.

Handling missing data.

Registry data that are often routinely collected bear the risk of incompleteness. In order to deal with this during data analyses, there are several options. Imputation, a statistical method that replaces missing data with substituted values, may be applied here. There are several imputation techniques, but multiple imputations that replace missing data by the average of the outcomes across multiple imputed data sets, is the most popular. The main advantage of multiple imputations is that sample size and variability is preserved. [31] The global PBC studies adjusted for missing data by multiple imputations, which did not affect the results. [18,19]

Privacy: Anonymous data entry.

Anonymous data entry in research is important, particularly for rare disease registries, as the patients may be traced back easily. According to privacy rules, the patient names should be substituted by specific codes. We used anonymous codes for all the PLD patients in our registry; and separate codes for their country and hospital. In order to trace back patients during follow-up, we use decoding lists for every center; including the research number, gender, birth date and hospital number. There needs to be caution taken to check the registries for double inclusion of patients. This can be performed by checking names; and if needed, data of patients with similar birth dates.

CONCLUSIONS

The use of registries in medical science clearly rises up to offer the opportunity to fill in important gaps in knowledge about rare diseases, through national and international collaboration. This papers provide a framework for the development of a clinical registry and includes the important aspects that need attention during this process.

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Chapter 5

Center is an important indicator for choice of invasive therapy in polycystic liver disease

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ABSTRACT

Polycystic liver disease (PLD) is a rare genetic disorder with progressive cyst growth as the primary phenotype. Therapy consists of volume reduction through invasive surgical or radiological procedures. To understand the process of treatment decision, our aim was to identify factors that increased the likelihood of treatment. We performed a cross-sectional study using an international population of patients with PLD. We collected data on the following therapies: liver transplantation, resection, fenestration and aspiration sclerotherapy. Data on the potential determinants, sex, center, autosomal dominant polycystic kidney disease (ADPKD), autosomal dominant polycystic liver disease (ADPLD), age at diagnosis, symptoms and phenotype were included. We corrected for follow-up time. We included 578 patients in our study, and 35% underwent invasive therapy. Multivariate regression analysis showed that number of symptoms and age at diagnosis of PLD increased the likelihood of treatment (respectively RR:1.4, $p<0.001$ and RR:1.4, $p=0.03$). The choice for liver transplantation or aspiration sclerotherapy was center-dependent (RR:0.7, $p<0.001$ and RR:1.1, $p=0.03$ respectively). The results of our international cross-sectional study suggests that a higher number of symptoms and every 10 years of PLD diagnosis increases the risk to undergo treatment by 40%. The choice to elect a particular modality is center dependent.

INTRODUCTION

Polycystic liver disease (PLD) is a condition characterized by progressive liver cyst growth. [1] PLD is part of the phenotype of two genetically distinct disorders. It is present as a primary phenotype in autosomal dominant polycystic liver disease (ADPLD), and it is the most common extra-renal manifestation in autosomal dominant polycystic kidney disease (ADPKD). [2-4] Isolated PLD is rare with a prevalence of 1 in 158.000, which contrasts that of ADPKD which has a higher prevalence (1: 400-1000 individuals). [5,6] The vast majority of ADPKD patients (94%) > 35 years-of-age possess hepatic cysts. [4]

Although PLD is often asymptomatic, patients who progress to severe hepatomegaly have a decreased health-related quality of life and are often in need of therapy. [7,8] Risk factors for progressive disease are age, female sex, estrogen use and pregnancies. [8-10] The concept central to PLD therapy is downsizing liver volume as this leads to improvement of symptoms. [3] Currently, the mainstay of therapies include invasive surgical and radiological procedures such as, liver transplantation, the only curative treatment, resection, fenestration and aspiration sclerotherapy.[2,11] Treatment is indicated in patients who suffer from symptomatic hepatomegaly and the choice for a specific therapy mainly depends on the presence and location of dominant cysts. [2,3,12] Clear guidelines about timing and choice of therapy are lacking. This might be explained by a lack of evidence generated by clinical trials comparing efficacy of invasive treatment strategies for PLD. In addition, there are no standardized outcome measures to assess treatment success in clinical practice, which hampers the building of an evidence base. In order to guide physicians on therapy, it is necessary to explore factors involved in the process of treatment decision. To this end, we aimed to delineate patient characteristics and disease-specific factors that trigger therapy. Our secondary aim was to identify determinants that increased the likelihood of a specific invasive therapy for PLD.

MATERIALS AND METHODS

Study design and subjects

We performed a cross-sectional study that included PLD patients coming from two independent PLD registries. Both registries were developed at two nationwide referral hospitals for PLD, Radboud university medical center Nijmegen in the Netherlands (center 1) and University Hospital of Leuven in Belgium (center 2). [13] Both hospitals are national referral centers for clinical evaluation and treatment of PLD. The Dutch registry contains all PLD patients who have visited the outpatient clinic of the Department of Hepatology of the Radboud university medical center between January 2008 and February 2015. Patients with ADPKD who had visited the Department of Nephrology between January 2008 and December 2014 were included, if PLD was present. The Belgium registry includes all PLD patients who had visited the

outpatient clinic of the Department of Hepatology of the University Hospital of Leuven, from January 2008 to July 2015. According to the Dutch and Belgium regulations, these registries do not need formal ethical approval as this was an observational study.

The inclusion criterium for this analysis was a diagnosis of PLD as documented by patients' physician where diagnosis of PLD had literally to be written down in the medical file of patients, or as shown on radiological imaging by the presence of ≥20 hepatic cyst larger than 0.5 cm. Underlying diagnosis of ADPKD or ADPLD was a requirement. ADPKD diagnosis was based upon modified Ravine criteria [14], and ADPLD diagnosis was based on the presence of ≥ 20 liver cysts, in the absence of renal cysts. If renal cysts were present in patients with ADPLD, the Ravine criteria should not be met. [15] Patients with hepatic cysts due to other diseases (e.g. Caroli's disease, autosomal resistant polycystic kidney disease) were excluded.

Potential determinants for treatment

We selected the following patient characteristics and disease-specific factors as potential determinants for treatment: sex, age at PLD diagnosis, phenotype, underlying diagnosis of PLD (ADPKD or ADPLD), number of symptoms, total liver volume (TLV), height adjusted TLV (hTLV), estrogen use and history of pregnancy. Center was also added as a factor determining choice of treatment. Parameters that pertained treatment decision were chosen on the basis of expert opinion, and evidence coming from studies on risk factors for severe PLD. [5,9,16]

Data collection

Data were retrospectively collected from medical charts of patients. We included information on the following invasive treatment modalities: liver transplantation (combined with or without renal transplantation), resection, fenestration and aspiration sclerotherapy. Experimental therapies such as somatostatin analogues were excluded since these drugs are mainly used in clinical trial settings. We reviewed medical charts for demographics, underlying diagnosis of PLD, age at diagnosis of PLD, TLV, hTLV, estrogen use and pregnancies. We used the most recent value for TLV that was available. Liver volumes had been calculated in the past by 3D measurements of CT scans. This included manually outlining of the liver every 9 mm with interpolation of intermediate slices and calculation of TLV. We also collected data on hepatic cyst phenotype by assessing MRI, CT, ultrasound images or reviewing imaging reports. We have distinguished between a phenotype with either the presence or absence of one or more dominant cysts (≥ 8cm). Finally, we collected data on the presence of the following symptoms in medical records of patients: abdominal discomfort, feeling full, abdominal pain, tiredness, pain in the rib cage and pain in the side. These symptoms were selected as they were overrepresented in a Dutch population of symptomatic PLD patients. [17]

Statistical analysis

We performed descriptive statistical analyses to summarize population characteristics. Baseline continuous variables were expressed in mean [standard deviation (SD)] for normally

distributed data or median [interquartile range (IQR)] for non-normally distributed data. Dichotomous outcomes were expressed as % (*n*/*n* total).

For our primary and secondary aim, we used multivariate logarithmic linked modified Poisson regression analysis to generate risk ratios (RRs) for determinants associated with respectively treatment and specific treatment modalities. [18,19] Risk ratios >1 and <1 were interpreted as respectively, increasing and decreasing the likelihood of treatment or a specific therapy, while a risk ratio equal to 1 with a 95% CI smaller than 1 indicates no association We included a potential determinant in the regression model only if at least 10 patients (1.7% of 578 patients) were exposed to the determinant to guarantee adequate statistical power. For the primary analysis, the dependent variable was specified as treatment or no treatment, whereas treatment was defined as patients who underwent surgical or radiological therapy, at least once. Independent variables included sex, center, age at diagnosis of PLD (defined as age at diagnosis of PLD divided by 10 years), phenotype, underlying diagnosis (ADPKD or ADPLD), number of symptoms, TLV, hTLV, estrogen use and pregnancy in history. We added the variable follow-up, defined as interval from diagnosis to inclusion in the study divided by 10 years to the model in order to correct for follow-up period.

For our secondary analysis, liver transplantation, resection, fenestration and aspiration sclerotherapy were included as dependent variables. In this analysis, the same independent variables as for the primary analysis were included. Unpaired Student's *t*-test or chi-square test were used to compare patient- or disease characteristics between specific treatment modalities. In addition, a sub-group analysis of hepatic phenotype and treatment strategy between ADPKD and ADPLD patients was performed. Finally, we tested whether patients who underwent aspiration sclerotherapy or fenestration differed on sex, center, age at diagnosis of PLD, phenotype and underlying diagnosis of PLD. If patients underwent aspiration sclerotherapy and fenestration, the first treatment that was given was used for this analysis. A *p*-value of <0.05 was considered statistical significant. Data were analyzed using SPSS 22.0 (SPSS Statistics, Inc., Chicago, IL, USA).

RESULTS

Characterization of the study population

We included 578 patients in our study population and 200 (35%) underwent invasive therapy (Fig. 1). The large majority of patients were female (81%) and 383 (66%) had an underlying diagnosis of ADPKD (Table 1). A total of 421 patients showed a phenotype without dominant cysts while a phenotype with concomitant dominant cysts was present in 54 patients. Liver phenotype significantly differed between ADPKD and ADPLD patients (Fig. 2). Patients with ADPLD possessed dominant cysts on radiological imaging in 34 patients (22%) whereas this was the case in only 21 of ADPKD patients (7%) (p <0.001).

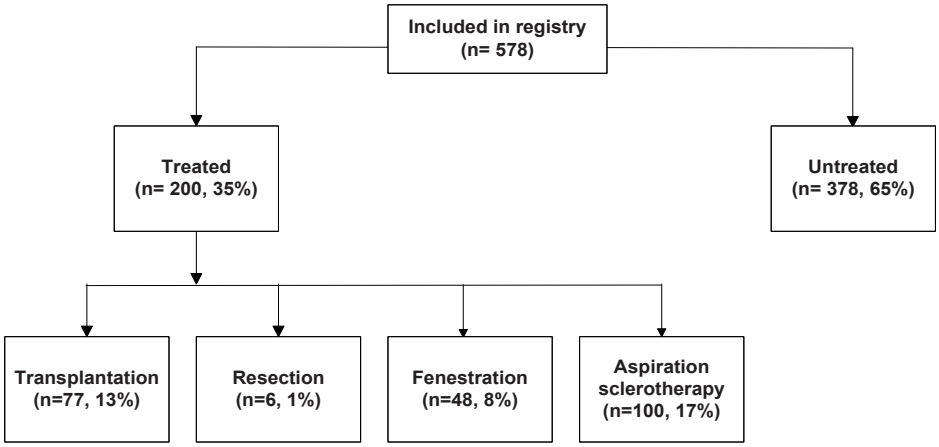

Figure 1. Overview of the study population. A total of 578 patients were included and 35% (n=200) received therapy. A number of patients received ≥ 1 treatment modality.

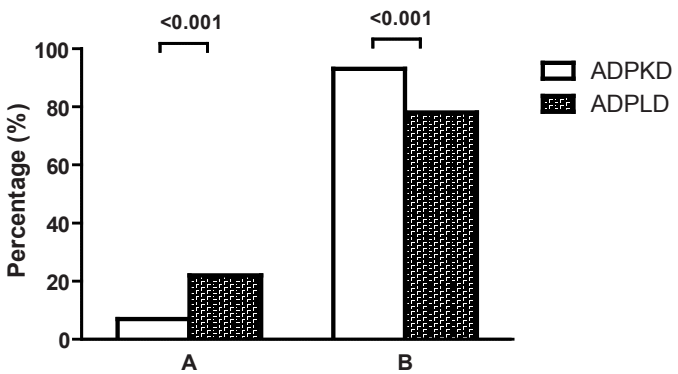

Figure 2. Liver cyst phenotype in ADPKD and ADPLD patients. (A) A phenotype with dominant cysts (≥ 8 cm) was significantly more present in ADPLD patients compared to ADPKD patients (22% vs. 7%, p <0.001). **(B)** A phenotype without dominant cysts was more often present in ADPKD (93% vs. 78%, p <0.001).

Most patients were Dutch (66%, n=380) and stratification of patients by center demonstrated that populations from both centers were comparable with respect to sex, TLV and hTLV (Table S1). Dutch patients had more symptoms, were more often diagnosed with ADPLD, diagnosed at a later age, and more often possessed a phenotype without dominant cysts. The follow-up of patients at the university hospital of Leuven in Belgium was significantly longer than the follow-up of patients at the Radboudumc in the Netherlands (18 vs. 7 years, *p* <0.001). Therefore, all analysis were corrected for follow-up time by including this in the multivariate model.

Table 1. Characteristics of the study population

	Complete population (n=578)	
Female	468	81%
ADPKD	383	66%
Age diagnosis PLD	45	±13
Center 1	380	66%
Volumetry*		
TLV (mL)	4093	[2717-6066]
hTLV (mL/m)	2639	[1669-3840]
Phenotype*		
≥ 20 cysts + ≥1 dominant cyst	54	11%
≥ 20 cysts	421	89%
Symptoms		
Abdominal tension	225	39%
Feeling full	210	36%
Abdominal pain	141	24%
Tiredness	129	22%
Pain rib cage	120	21%
Pain side	78	14%
No of symptoms	1	[1-3]

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; hTLV, height adjusted total liver volume; PLD, polycystic liver disease; TLV, total liver volume.

Date are in number and percentage (%), mean ±standard deviation, or median and interquartile range [IQR]. Center 1= Radboud university medical center Nijmegen, the Netherlands, center 2= University Hospital of Leuven, Belgium

*Data were missing in respectively 56%, 72% and 18% of patients for TLV, hTLV and phenotype

Determinants that trigger treatment

Liver transplantation, resection, fenestration and aspiration sclerotherapy were performed in respectively 13%, 1%, 8% and 17% of patients (Fig. 1). Multivariate regression analysis revealed that patients who suffer from more symptoms have a 40% higher likelihood (CI: 1.17-1.60, p<0.001) to receive treatment (Fig. 3). Every 10 years of diagnosis of PLD was also significantly associated with a 40% increased risk of treatment (CI: 1.04-1.88, p=0.03)). . TLV, hTLV, estrogen use and pregnancy in history were not included in the regression analyses as data were missing in respectively 56%, 72%, 54% (women only), and 49% (women only) of patients.

Characterisation of patient undergoing invasive therapy

Table S3. provides an overview of patient and disease characteristics of individuals who underwent respectively transplantation, resection, fenestration and aspiration sclerotherapy. Liver transplantation was carried out at a median age of 53 ±10 years in a total of 77 patients of whom 69 were diagnosed with ADPKD. In 43% (n=33) of patients this was combined with a renal transplantation and radiological imaging showed a median TLV of 4271 mL [IQR 3438-6243 mL] in these patients. Resection was done in 6 patients (1%) and most patients underwent other treatment modalities as well. Interestingly, a total of 27 patients received >1 different treatment modality. A combination of fenestration and aspiration sclerotherapy was most common (9%, n=17). Only a small proportion of the total study population underwent fenestration (8%, n=48) and a minority of them had ADPKD (38%, n=18). Patients who underwent fenestration or aspiration sclerotherapy did not significantly differ on sex, diagnosis, age at diagnosis and phenotype (Table S4). We found that the choice for fenestration or aspiration sclerotherapy was mainly center dependent (p<0.001). Aspiration sclerotherapy was most frequently performed with a total of 197 procedures in 100 patients. Half of patients (49%, n=97) had > 1 procedure (range 1-15 procedures), and the majority of patients were diagnosed with ADPLD (65%, n=65).

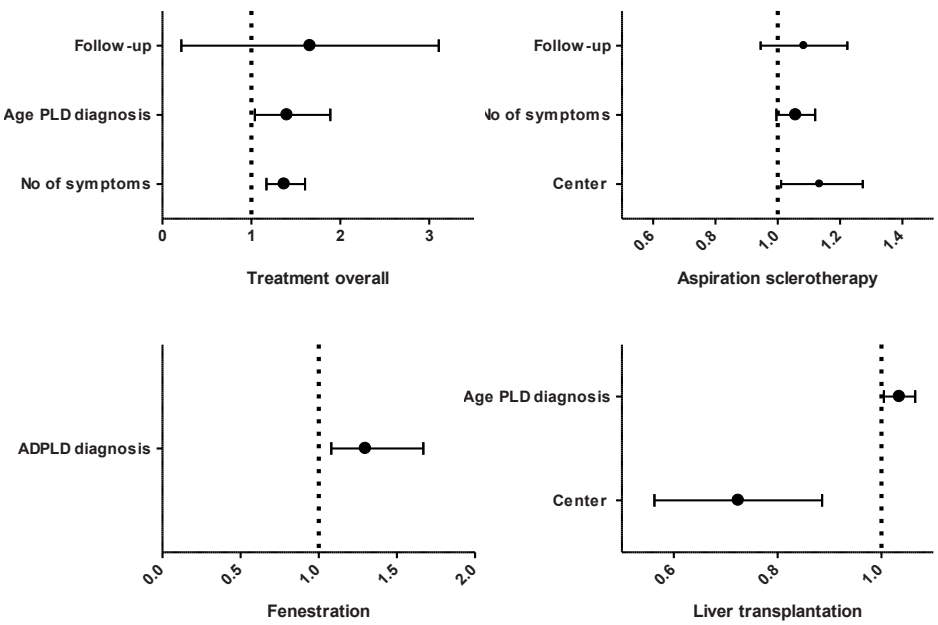

Figure 3. Forest plots showing risk ratios with 95% confidence intervals for respectively treatment in general, liver transplantation, fenestration and aspiration sclerotherapy. Risk ratios were calculated by multivariate regression analyses. A risk ratio of <1 represents a decreased risk and >1 increased risk for treatment. A risk ratio of 1 (dotted line) indicates no association.

Determinants associated with invasive therapies

We tested the association of six potential determinants with the likelihood to undergo either liver transplantation, fenestration or aspiration sclerotherapy (Fig. 3, Table S5). Determinants associated with hepatic resection were not analyzed as only seven patients (1%) underwent this procedure and a minimum of 10 patients was required. Patients from center 2 had a 30% higher likelihood (p<0.001) to receive a liver transplantation compared to patients from center 1 (Table S5). .The likelihood to undergo a liver transplantation increased by 4% with every 10 years of PLD diagnosis (RR 1.035, CI: 1.005-1.065). Multivariate analysis revealed that a diagnosis of ADPLD increased the likelihood to undergo fenestration by 30% (p<0.05). The number of symptoms was associated with a higher likelihood to undergo aspiration sclerotherapy (RR 1.1, p<0.001) . The likelihood to be subjected to aspiration sclerotherapy was center dependent (RR 1.1, p=0.03) and increased by follow-up time (RR=1.1, P=0.002).

Therapy differs between patients with ADPKD and ADPLD

We subsequently explored whether treatment strategies differed between patients with ADPKD and ADPLD. Fenestration and aspiration sclerotherapy were significantly more often performed in patients with ADPLD than in patients with ADPKD (15% vs. 5% and 33% vs. 9%, both p<0.001) (Fig. 4). By contrast, patients with ADPKD more frequently underwent liver transplantation (18% vs. 4%, p<0.001). In a total of 33 patients undergoing liver transplantation, this was combined with a renal transplantation on the same day.

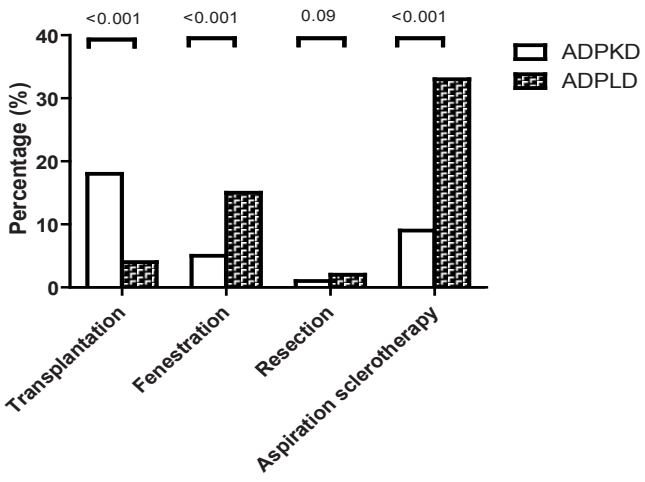

Figure 4. Treatment strategies for PLD in autosomal dominant polycystic kidney disease (ADPKD) and autosomal dominant polycystic liver disease (ADPLD). Liver transplantation was more often done in ADPKD patients whereas fenestration and aspiration sclerotherapy favored ADPLD patients.

DISCUSSION

The results of our large cross-sectional study demonstrate that number of symptoms and age at diagnosis of PLD increased the likelihood to receive treatment for PLD. The choice for either liver transplantation or aspiration sclerotherapy was center-dependent. This underscores a certain arbitrariness, probably due to a lack of evidence that supports any of the available treatment options.

A previous review suggests that the natural course of PLD in ADPLD and ADPKD is similar. [3] Our study suggests otherwise, and demonstrates that ADPLD patients more often possessed large dominant cysts, a phenotype that is amendable to treatment with fenestration or aspiration sclerotherapy. [3] The available therapies for patients without dominant cysts, most often patients with ADPKD, are resection or liver transplantation. [2,3] A resection is a high risk procedure and not often performed in our population. Liver transplantation is a very invasive procedure, in particular for a disease that does not lead to liver failure or death. In view of a lack of donors, it is not offered widely. [2,3] These reasons probably explain why only 13% of this severe PLD population was transplanted while a higher percentage is in need of curative therapy. Interestingly, 90% of the transplanted patients were female. This might be explained by a more severe disease course in women, probably due to the effect of estrogen. [9] However, diagnosis was not significantly associated with any of the invasive therapies. These results are in contradiction with a cohort study showing that aspiration sclerotherapy was significantly more performed in patients with ADPLD, while patients with ADPKD were more often considered for liver transplantation. [20]

About 14% of our treated study population underwent subsequent treatments. The combination of fenestration and aspiration sclerotherapy was the most frequently chosen option. In view of the comparable patients‘ characteristics, our data lend support to center specific decision making when it comes to the choice between both treatment modalities. These findings indicate that available expertise drives treatment while evidence that singles out the best treatment modality is lacking. A randomized controlled trial comparing effectiveness of fenestration and aspiration sclerotherapy is necessary to find out which treatment strategy is most effective. At the minimum, we should assess treatment outcomes in clinical care of PLD in an uniform fashion. Assessment of TLV, symptoms and health-related quality of life before and after treatment should become standard of care and collected in a registry to build an evidence base of treatment efficacy. [21] In addition, our results demonstrated that liver transplantation was more frequently performed in Belgium while in the Netherlands there was a preference for aspiration sclerotherapy. Due to a different organ donor policy, Belgium has more donors available. This might lower the threshold for physicians to offer transplantation as a treatment option.

Our study also discovered that a large proportion of patients received treatment (35%), which is at odds with literature that indicates that only a small subset of patients is symptomatic. [2,3,11] Our population consists of patients referred to two nationwide tertiary referral centers, which may have contributed to a selection of a population with more severe disease. This may overestimate the disease burden of PLD in this population as referral to these centers is often triggered by presence of symptoms. The threshold to treat PLD patients is probably lower in tertiary centers because of wider experience with PLD and its treatment options.

The strength of our study was the large, international study population (n=578) in view of the rarity of PLD. In addition, both patients with ADPKD and ADPLD were represented in this study as well as the most prevalent surgical therapies. Patients from all over the country are referred to one of both centers and therefore this study provides a good overview of the clinical profile of treated patients in both countries and made it possible to study the effect of center on treatment decision.

The main limitation of our study is the cross-sectional design. We were able to investigate factors involved in treatment decision, but it is impossible to infer causality. The retrospective data collection has led to missing data and therefore liver volume could not be included as potential determinant in our prediction model. Assessment of liver volume is time consuming and trained staff and software is required. This might explain the amount of missing data for TLV. Although, it is questionable whether liver volume plays a major role in the treatment decision process as so little volumes were available in the medical charts. Therefore this probably had no major impact on the primary outcome. This registry will continue in a prospective fashion including more patients worldwide with a long-term follow up. A prospective nature will decrease the number of missing data and the long-term follow-up will support to learn more about the prognosis of PLD.

CONCLUSION

The results of our international cross-sectional study suggests that a higher number of symptoms and every 10 years of PLD diagnosis increases the likelihood to undergo treatment by 40%. The choice to elect a particular modality is center dependent. The major implication of these findings is that physicians should bear in mind that there is no evidence that favors either treatment option, and this contributes to center specific preferences. Therefore, assessing efficacy of therapy by measuring liver volume and symptom burden, are essential to gain evidence among the best treatment option. Future studies comparing efficacy of treatment modalities would be helpful to fill the gap of knowledge among the best treatment option.

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SUPPLEMENTARY FILES

Table S1. Characteristics of the study population split for center

	Center 1 (n=380)		Center 2 (n=198)		p
Treatment	121	32%	79	40%	0.05
Sex (% females)	305	80%	163	82%	0.55
Diagnosis (% ADPKD)	230	61%	153	77%	<0.001
Age diagnosis PLD	47	±12	39	±15	<0.001
TLV (mL)	4324	[2795-6459]	3520	[2543-5292]	0.07
hTLV (mL/m)	2736	[1683-3916]	2163	[1663-3106]	0.32
Phenotype (% ≥ 20 cysts)	296	90%	125	84%	0.04
No of symptoms	2	[0-3]	1	[0-2]	<0.001
Follow-up (yrs.)	7	[3-13]	18	[10-32]	<0.001

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; hTLV, height-adjusted total liver volume; PLD, polycystic liver disease; TLV, total liver volume.
Date are in number and percentage (%), mean ± standard deviation, or median and interquartile range [IQR].
Center 1= Radboud university medical center Nijmegen, the Netherlands, center 2= University Hospital of Leuven, Belgium

Table S2. Regression model including potential determinants of treatment

	Multivariate regression analysis		
Variables	**RR**	**(95% CI)**	***p***
Female sex	1.8	(0.76-4.28)	0.18
Center 1 vs. 2*	-0.6	(0.28-1.30)-	0.20-
Age diagnosis PLD	**1.4**	**(1.04-1.88)**	**0.03**
Phenotype 1 vs. 2**	1.0	(0.36-2.86)	1.0
ADPLD	1.2-	(0.17-9.30)-	0.8-
No of symptoms	**1.4**	**(1.17-1.60)**	**<0.001**
Follow-up	**1.6**	**(1.11-2.27)**	**0.01**

Abbreviations: ADPLD, autosomal dominant polycystic liver disease; PLD, polycystic liver disease; RR, relative risk.
*Center 1= Radboud university medical center Nijmegen, the Netherlands, center 2= University Hospital of Leuven, Belgium
**Phenotype 1= ≥ 20 cysts, phenotype 2= ≥ 20 cysts with ≥ 1 dominant cyst

Table S3. Treatment strategies in PLD patients

	Transplantation (n = 77)		Resection (n=6)		Fenestration (n = 48)		Aspiration sclerotherapy (n=100)	
Number of procedures	77	-	7	-	54	-	197	-
Sex (% females)	66	(86%)	5	(83%)	45	(94%)	88	(88%)
Diagnosis (% ADPKD)	69	(90%)	2	(33%)	18	(38%)	35	(35%)
Mean age diagnosis PLD	40	±12	41	±3	44	±11	47	±13
Mean age treatment	53	±10	49	±9	50	±13	53	±12
Center 1*	7	(9%)	3	(50%)	33	(69%)	194	(98%)
Liver volume**								
TLV (mL)	4271	[3438-6243]	-	-	4122	[2436-5890]	3921	[2835-5445]
hTLV (mL/m)	4340	[2402-4884]	-	-	2635	[1522-3743]	2367	[1678-3272]
Phenotype (% ≥ 20 cysts)**	47	(98%)	4	(100%)	30	(81%)	69	(75%)
Other therapies								
Hepatic resection	1	(1%)	1	(17%)	3	(6%)	2	(2%)
Aspiration sclerotherapy	5	(7%)	2	(33%)	17	(35%)	49	(49%)
Fenestration	8	(10%)	3	(50%)	6	(13%)	17	(17%)
Transplantation	0	(0%)	1	(17%)	8	(17%)	5	(5%)
Combined with RT	33	(43%)	0	(0%)	2	(4%)	3	(3%)

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ADPLD, autosomal dominant polycystic liver disease; hTLV, height adjusted total liver volume; TLV, total liver volume; PLD, polycystic liver disease; RT, renal transplantation.

Date are in number and percentage (%), mean ± standard deviation, or median and interquartile range [IQR].

*Center 1= Radboud university medical center Nijmegen, the Netherlands.

**TLV was missing in respectively 33%, 50%, 50% and 69% of patients. hTLV was missing in respectively 46%, 67%, 67% and 91% patients. Phenotype was missing in respectively 8%, 23%, 33% and 38% of patients.

Table S4. Comparison of patients who underwent aspiration sclerotherapy or fenestration

	Aspiration sclerotherapy (n=84)		**Fenestration (n=37)**		
	n	**%**	**n**	**%**	***p***
Female sex	72	(86%)	34	(92%)	0.34
ADPLD	53	(63%)	22	(59%)	0.70
Age diagnosis PLD	48	±14	45	±12	0.34
Phenotype 1 vs. 2*	60	(71%)	24	(65%)	0.35
Center 1**	82	(98%)	24	(65%)	<0.001

Abbreviations: ADPLD, autosomal dominant polycystic liver disease; PLD, polycystic liver disease.

Date are in number and percentage (%) or mean ± standard deviation.

*Phenotype 1= ≥ 20 cysts, phenotype 2= ≥ 20 cysts with ≥ 1 dominant cyst

**Center 1= Radboud university medical center Nijmegen, the Netherlands

Table S5. Regression model showing factors associated with selection of treatment modality for PLD

	Multivariate regression analysis								
	In favor of transplantation			In favor of fenestration			In favor of aspiration sclerotherapy		
Variables	**RR**	**95% CI**	***p***	**RR**	**95% CI**	***p***	**RR**	**95% CI**	***p***
Female sex	1.1	(1.00-1.15)	0.09	1.0	(0.96-1.08)	0.6	1.0	(0.94-1.15)	0.5
Center*	**0.7**	**(0.68-0.80)**	**<0.001**	1.0	(0.93-1.07)	0.9	**1.1**	**(1.01-1.27)**	**0.03**
Age diagnosis PLD	**1.0**	**(1.00-1.01)**	**0.02**	1.0	(1.0-1.0)	0.9	1.0	(0.99-1.08)	0.1
Phenotype 1 vs. 2**	-	-	-	1.0	(0.94-1.16)	0.4	0.9	(0.72-1.02)	0.08
No of symptoms	1.0	(0.99-1.02)	0.63	1.0	(0.99-1.02)	0.4	**1.1**	**(1.03-1.08)**	**<0.001**
ADPLD	0.9	(0.70-1.15)	0.4	**1.3**	**(1.08-1.67)**	**<0.05**	0.9	(0.62-1.26)	0.5
Follow-up	1.0	(1.00-1.01)	0.08	1.0	(1.0-1.0)	1.0	**1.1**	**(1.03-1.14)**	**<0.05**

Abbreviations: ADPLD, autosomal dominant polycystic liver disease; PLD, polycystic liver disease; RR, relative risk.

*Center 1= Radboud university medical center Nijmegen, the Netherlands, center 2= University Hospital of Leuven, Belgium

**Phenotype 1= ≥ 20 cysts, phenotype 2= ≥ 20 cysts with ≥ 1 dominant cyst

Part III

A novel drug for polycystic liver disease

Chapter 6a

Rationale and design of the CURSOR trial: An international, multicenter, randomized controlled phase II clinical trial to study the effect of ursodeoxycholic acid as a volume reducing treatment for symptomatic polycystic liver disease

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ABSTRACT

Background and aims

A minority of patients with polycystic liver disease suffer from symptomatic hepatomegaly. Conventional invasive therapies may cause serious complications and have high recurrence rates. Somatostatin analogues can decrease liver volume by diminishing increased intracellular cAMP levels in polycystic cholangiocytes, but show modest therapeutic benefit and have considerable side effects. The bile acid ursodeoxycholic acid is an endogenous bile acid and FDA-approved for the treatment of primary biliary cirrhosis. Experimental evidence suggests that ursodeoxycholic acid reduces cystogenesis in an experimental model of polycystic liver disease by restoring diminished intracellular free calcium levels in polycystic cholangiocytes and by removing cystic accumulation of cytotoxic bile acids. We hypothesize that ursodeoxycholic acid may be effective in reducing total liver volume in polycystic liver disease patients.

Methods

This international, multicenter, randomized, controlled phase II clinical trial evaluates the effect of 24 weeks of ursodeoxycholic acid administration as a liver volume reducing treatment for polycystic liver disease. Eligible patients are symptomatic polycystic liver disease patients that have a total liver volume ≥ 2500 mL. A total of 34 patients will be randomized through a 1:1 allocation. The intervention group will receive ursodeoxycholic acid 15-20mg/kg/day for 24 weeks, the control group will receive standard care. Primary outcome is change in total liver volume determined by computer tomography volumetry. Secondary outcomes are change in symptoms and health-related quality of life measured by questionnaires and change in laboratory markers like alkaline phosphatase and -glumatyltransferase. Moreover, safety and tolerability of the drug will be assessed.

Discussion

Treatment of polycystic liver disease with ursodeoxycholic acid appears promising based on experimental observations. A 4% difference in total liver volume in favor of ursodeoxycholic acid treatment versus standard care would indicate comparable efficacy of ursodeoxycholic acid to that of somatostatin analogues at lower costs, less side effects and contra-indications and a more acceptable route of admission. In case of a positive result, further research should focus on the molecular mechanism of action of ursodeoxycholic acid in polycystic liver disease, dose-response relationship and combination therapy of somatostatin analogues with ursodeoxycholic acid to evaluate synergistic effects.

Trial registration

This trial is registered with ClinicalTrials.gov (identifier: NCT02021110) and EudraCT (identifier: 2013-003207-19)

BACKGROUND

Polycystic liver diseases (PLD) are genetic disorders characterized by the formation of multiple cysts in the liver derived from the biliary cells (i.e. cholangiocytes). PLD can occur in combination with renal cysts as a manifestation of autosomal dominant polycystic kidney disease (ADPKD), or without renal cysts as autosomal dominant polycystic liver disease (ADPLD). [1] PLD can lead to symptoms such as abdominal pain, dyspnea and early satiety due to the enlarged liver. Furthermore physical health-related quality of life (HRQL) is significantly lower in PLD patients compared to the general population. [2] The natural course of PLD dictates a growth of 1.8% in 6-12 months. [1-3] This progressive hepatic cystogenesis in PLD is a consequence of increased levels of cAMP and decreased levels of intracellular calcium in cholangiocytes. [4-7] Somatostatin analogues decrease cAMP levels and have shown to reduce total liver volume (TLV) with ~5% in 6-12 months. [3, 8-10] Although successful to some extent, therapy with somatostatin analogues have some inherent disadvantages. A proportion of patients does not respond to somatostatin analogues or has side effects such as glucose intolerance, diarrhea or development of gall stones. Finally, in most health care systems treatment with somatostatin analogues is very expensive. [11] Therefore other options are needed.

Ursodeoxycholic acid (UDCA) is a hydrophilic endogenous bile acid that is FDA-approved for the treatment of several cholestatic disorders such as primary biliary cirrhosis (PBC).[12-14] In vitro studies showed that polycystic human cholangiocyte cultures are characterized by decreased [Ca^{2+}] compared to normal human cholangiocytes. UDCA increased intracellular calcium and blocked cholangiocyte hyperproliferation in a dose-dependent manner. These effects were also observed in cystic cholangiocytes isolated from the PCK rats, an animal model that recapitulates human PLD. UDCA treatment for five months inhibited hepatic cystogenesis, fibrosis, inflammation, and improved physical status in PCK rats. Moreover, PCK rats showed increased intrahepatic concentration of bile acids (particularly within the cystic fluid) compared to normal rats. Moreover, the bile that exit the liver of PCK rats presented decreased concentration of bile acids compared to normal rats. UDCA treatment decreased the concentration of the most cytotoxic bile acids from the liver of PCK rats and normalized the bile acid concentration in bile. [15]

Based on these results, we hypothesize that chronic UDCA treatment may be an effective therapy in reducing TLV, reducing symptoms and increasing HRQL in PLD patients.

Therefore we designed an international, multicenter, randomized controlled phase II clinical trial to assess whether UDCA [1] is effective in reducing TLV in symptomatic PLD patients, [2] improves symptoms and HRQL and [3] is safe and well tolerated.

METHODS/DESIGN

Study aim

The primary aim of the CURSOR is to determine whether UDCA is effective in reducing TLV in symptomatic PLD patients with liver volumes ≥ 2500 mL. PLD patients suffering from ADPKD or ADPLD will receive UDCA 15-20 mg/kg/day for 24 weeks (Appendix A. Fig. 1.). Secondary objectives are to determine change in symptoms and HRQL, and change in laboratory values like alkaline phosphatase (AP) and -glutamyltransferase (GGT). Finally, safety and tolerability of UDCA treatment will be assessed.

Hypothesis

We hypothesize that ursodeoxycholic acid may be effective in reducing total liver volume in PLD patients.

Study design and setting

The CURSOR is an international, multicenter, randomized controlled phase II clinical trial in PLD patients. Patients will be recruited through 3 specialized centers for PLD; one in Spain (Donostia University Hospital, San Sebastian, Spain) and two in the Netherlands (Academic Medical Centre Amsterdam and Radboud university medical center, Nijmegen). Trial duration will be 40 weeks, consisting of a screening period (4 weeks), treatment period (24 weeks) and follow-up (12 weeks). At start and end of treatment we will measure TLV in all patients by CT-scanning . Total kidney volume (TKV) in ADPKD patients will be assessed as the natural course can be an important factor and possible confounder within the relation between TLV and symptoms or HRQL. In addition, all patients will be evaluated at week 4 and 12 for adverse events, drug compliance and lab chemistry. We will complete drug accountability logs and record vital signs. Inclusion has started in May 2014 and was completed in February 2015. Figure 1. depicts a schematic overview of the trial design.

Randomization and treatment allocation

This trial aims to include 34 PLD patients. Eligible patients will be randomized through a 1:1 allocation. The intervention group will receive UDCA daily (15-20mg/kg/day), the control group will receive standard care. Randomization will be performed using block randomization with a block size of four. Patients will be assigned a randomization number which corresponds to a treatment arm concealed in a closed envelop. Randomization will be performed by an independent researcher.

Study population

Symptomatic PLD patients between 18 and 80 years with underlying diagnosis of ADPLD or ADPKD and TLV ≥ 2500mL are eligible for participation to this study. ADPKD diagnosis is based upon modified Ravine[16] criteria and PLD is defined as the presence of > 20 liver cysts on CT

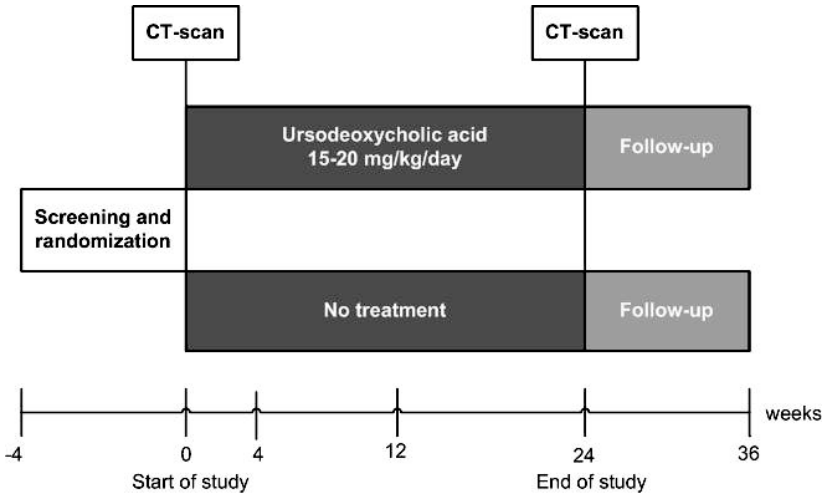

Figure 1. Trial design of the CURSOR trial. All patients are screened for eligibility and the patients who suit the criteria are randomized in an equal ratio to either the UDCA arm or the control arm. All patients receive a CT scan at baseline and after 24 weeks of treatment. Control visits are performed at week 4, 12 and 24 after baseline. Follow-up visit will be performed 12 weeks after end of study.

or MRI scan. TLV has to be judged by the researcher and can be based on clinical findings (hepatomegaly), former scans or TLV measurements in the past.

Symptomatic is defined as Eastern Cooperative Oncology Group – Performance Status (ECOG-PS) ≥ 1 [17], and having at least three of the following symptoms:

- Abdominal pain
- Abdominal distension
- Abdominal fullness
- Dyspnea
- Early satiety
- Back pain
- Nausea/vomiting
- Anorexia
- Weight loss
- Jaundice

The specific study inclusion and exclusion criteria are listed below.

Inclusion criteria

- 18 ≤ age ≤ 80 years
- PLD, defined as > 20 liver cysts on CT or MRI scan, with underlying diagnosis of ADPLD or ADPKD
- TLV ≥ 2500 mL
- Symptomatic defined as ECOG-PS ≥ 1 [17], and having at least three out of ten PLD symptoms
- Signed informed consent

Exclusion criteria

The following exclusion criteria will be assessed by questioning the patient and assessing patient his medical file.

- Use of oral contraceptives or estrogen supplementation
- Use of UDCA in the three months before baseline
- Females who are pregnant or breast-feeding or patients of reproductive potential not employing an effective method of birth control.
- Intervention (aspiration or surgical intervention) within six months before baseline
- Treatment with somatostatin analogues within six months before baseline
- Renal dysfunction (estimated glomerular filtration rate calculated by the
- Modification of Diet in Renal Diseases (MDRD) < 30 ml/min/1.73m^2)
- Patients with a kidney transplant
- Hypersensitivity reaction to UDCA or patients with galactose-intolerance, lactase deficiency or glucose-galactose malabsorption
- Acute cholecystitis or frequent biliary colic attacks
- Acute stomach or duodenal ulcers
- Inflammation of small intestine or colon
- Use of drugs that can interact with UDCA, such as colestyramine, aluminium hydroxide or cyclosporine
- Enrolment in another clinical trial of an investigational agent while participating in this study

The following exclusion criteria will be judged by the investigator screening the patient for eligibility:

- History or other evidence of severe illness or any other conditions which would make the patient, in the opinion of the investigator, unsuitable for the study
- Mental illness that interferes with the patient ability to comply with the protocol

Trial treatment

The intervention group will receive an oral dosage of 15-20 mg/kg/day UDCA in 2 doses (morning and evening) for 24 weeks. UDCA is a non-toxic, endogenous, hydrophilic bile acid which affords protection against hydrophobic bile acids. UDCA is well accepted in clinical practice, physicians have experience with the drug for more than 30 years. For example, oral UDCA administration is safe and the only effective therapy approved by the FDA (13-15 mg/kg bodyweight per day) for PBC. [18] The mechanism of UDCA seems to be dose-dependent in PBC and therefore the highest acceptable dose is preferred to achieve the maximum effect. [19] This accords with in-vitro data that documented a dose dependent inhibition of UDCA on the hyperproliferation of polycystic human cholangiocytes. Moreover, doses of 25 mg/kg/day for 5 months showed therapeutic benefits in PCK rats. [15] As a result patients will receive a UDCA dose of 15-20mg/kg/day. The most common (1-10%) side effects are diarrhea and sticky stools. [20] In case of dose related side effects down titration will be accomplished by reducing the amount of pills, one pill at a time.

Study endpoints

Primary outcome

The primary objective of the CURSOR trial is to assess the proportional change in TLV from baseline to week 24.

Secondary outcomes

Secondary outcomes are as follows:

- Absolute change in TLV and TKV from baseline to week 24
- Proportion of patients having any reduction in TLV
- Proportional change in TKV from baseline to week 24
- Change in symptoms measured by the gastro-intestinal questionnaire (GI-Q) [21] PLD-questionnaire (PLD-Q), and EORTC QLQ-C30 at baseline, end of treatment and follow-up.
- Change in HRQL as measured by the Medical Outcomes Study 36-item Short-Form Health Survey (SF-36) and visual-analogue scale (VAS) score of the European Quality of Life-5 dimension (EQ5D) at baseline, end of treatment and follow-up.
- Change in laboratory markers like GGT and ALP at baseline, end of treatment and follow-up.
- Evaluation of safety and tolerability by documenting (serious) adverse events

Data collection

All data will be collected by a case record form designed to capture all visit information including medical history, medication use and adverse events. The duration of the trial will be 40 weeks for all patients. Screening will be completed in week -4 to 0, in the second phase patients are either treated with UDCA or receive standard care for 24 weeks. During this phase

patients will visit the hospital at baseline (week 0), week 4, 12 and 24 (end of treatment). Follow-up visit will be at week 36, 12 weeks after end of treatment. Medical history, medication use, adverse events, tolerability and drug accountability will be assessed during each visit. Furthermore vital signs, upper arm circumference and weight are measured. In addition blood samples will be drawn. The two main study visits at week 0 and 24 include a CT-scan and 5 questionnaires. At follow-up, the 5 questionnaires will be completed as well.

Study procedures

This study consist of six visits including screening visit, 3 treatment visits (week 4, 12, 24) and one follow-up visit (week 36). The requested parameters at the different visits are listed below.

Screening

- Written informed consent
- Eligibility criteria check
- Assessment of ECOG-PS score [17]
- Verification of the diagnosis of PLD
- Medical history and concomitant medication
- Physical examination and vital signs
- Upper arm circumference
- Laboratory chemistry: liver function tests (INR, albumin), liver enzymes (AST, ALT, total bilirubin, direct bilirubin, GGT, ALP, kidney function (MDRD)

Every visit

- Vital signs
- Weight
- Upper arm circumference
- Lab chemistry: Liver enzymes, kidney function, albumin
- Adverse events
- Concomitant medication
- Drug accountability

Baseline (week 0)

- Serum storage for future reference (6 ml)
- Questionnaires
  - GI-Q
  - SF-36 questionnaire
  - PLD-Q
  - EORTC QLQ-C30
  - VAS score- EQ5D
- CT scan of liver and kidneys
- Randomization and treatment allocation to UDCA or standard treatment

End of treatment (week 24)

- Serum storage for future reference (6 ml)
- Questionnaires
  - GI-Q
  - SF-36 questionnaire
  - PLD-Q
  - EORTC QLQ-C30
  - VAS score- EQ5D
- CT scan of liver and kidneys

Follow-up visit (week 36)

- Serum storage for future reference (6 ml)
- Questionnaires
  - GI-Q
  - SF-36 questionnaire
  - PLD-Q
  - EORTC QLQ-C30
  - VAS score- EQ5D

CT Scanning and 3-Dimensional Volumetry

CT scans at baseline and week 24 will be performed without contrast on a multidetector CT scanner (Somatom Sensation 64; Siemens Medical Solution AG, Erlangen, Germany). All CT scans are blinded to patient identity and date of birth as well as date of scan. TLV and TKV will be calculated by 3D measurement of CT scan slices using Pinnacle3 ® version 8.0 g (Philips, Eindhoven, The Netherlands). Imaging protocol includes that CT scans will have a slice thickness of 3 mm. Liver and separate kidneys will be outlined manually every 9 mm. The software interpolates the intermediate slices and calculates the areas within the indicated circumference, and finally, TLV and TKV. All CT scans will be measured by 2 researchers, TLV will be determined by using the average of both measurements. Inter-observer variation will be calculated. Unblinding of CT scans will be performed after measurement of volumes of all patients.

Patient reported outcome measures

During the CURSOR study patients will complete the GI-Q, the PLD-Q and the EORTC QLQ-C30 in order to assess symptoms. The GI-Q contains 11 questions about symptoms that patient have suffered from over the past four weeks using a 7-point Likert scale, ranging from 0 ("none") to 6 ("severe"). The PLD-Q is a recently developed questionnaire specific for PLD patients and contains 16 questions (PLD-Q, Radboud University Medical Center, Nijmegen, the Netherlands). As the PLD-Q has not been validated yet, the EORTC QLQ-C30, a validated questionnaire has been included in the CURSOR as well. This questionnaire includes 13 symptoms which can be scored from 1 ("not at all") to 4 ("very much"). [22]

Change in HRQL will be assessed by the SF-36 and VAS score-EQ5D. The SF-36 is a 36-questions instrument consisting of eight scales of health assessing functional health and well-being. These scales have been clustered into a physical component summary score (PCS), and a mental component summary score (MSC) and have been validated in a variety of studies for multiple chronic illnesses. [23]

The VAS score-EQ5D records the patient's self-rated health on a vertical, visual analogue scale where the endpoints are labeled "best imaginable health state" ("100") and "worst imaginable health state" ("0").

Laboratory values

Several laboratory values will be measured during the study timeline for safety measures. Abnormalities in levels of the liver enzymes AST and ALT in PLD patients are generally absent. However, serum levels of ALP and GGT are often raised in patients suffering from severe hepatomegaly due to PLD. [24] As UDCA seems to reduce these levels shown in former trials in PBC patients, secondary endpoint is to analyze the effect of UDCA on ALP and GGT compared to the control group. [25, 26]

Study withdrawal

All subjects have the right to withdraw from the study at any time during the trial. Patients will be withdrawn from the study for any of the following reasons: withdrawal of informed consent, pregnancy, failure to adherence to protocol requirements, study drug discontinuation, surgical intervention on the liver during the trial and if the investigators conclude that it is in the patient's best interest for any reason. Replacement after study withdrawal will only be allowed if withdrawal occurs between screening and baseline.

Sample size considerations

We think that in order for UDCA to be an effective therapy, it needs to be at least equally effective as somatostatin analogues. A recent trial showed a 4% difference in means in TLV between the somatostatin and placebo group. [3] A sample size of 30 will achieve 80% power to detect a difference of 4% in liver volume using a two-sided a-level of 0.05. Taken into account a dropout rate of 10%, the minimal sample size needs to be 34 patients with 17 patients in each group.

Statistical analysis

Intention-to-treat (ITT) analyses will be used for all clinical outcome variables. The ITT population includes all patients who received at least one dose of the study drug.

The effect of UDCA will be assessed by determining the proportional change between baseline and final CT-scan between the two groups. TLV will be determined as indicated before. Continuous variables will be expressed in mean ± SD if normally distributed, otherwise as median

± interquartile range. All secondary outcomes will be compared between both arms using *t* test for normally distributed data and the Mann-Whitney U for non normally distributed data. Symptom scores of the EORTC

and HRQL measured by the SF-36 and VAS-score EQ5D, will be compared between both treatment arms and calculated by using the appropriate scorings manuals. [22, 23, 27] Change in symptom severity as measured by the GI-Q will be calculated by using a sum score of upper gastro-intestinal symptoms severity ranging from 0 to 66. [21, 28]

All adverse events occurring during the study will be recorded in the patient's medical records. The incidence of events considered to be at least possibly related to the study treatment will be summarized by treatment group and severity.

All statistical analyses will be two-sided with a critical significance level of 5%. Analyses will be performed using SPSS software version 20.0 (Chicago,IL,USA).

Ethical considerations

Ethical approval has been obtained from the Central Committee on Research Involving Human Subjects and by the local accredited Medical Research Ethics Committee of the region Arnhem-Nijmegen, the Netherlands (reference number: 2013-371) . This study will be performed in accordance with the protocol, the guidelines of Good Clinical Practice/ ICH, the principles of the Declaration of Helsinki 1964 as modified by the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000 including two notes of clarification paragraph 29 and 30, and the local national laws governing the conduct of clinical research studies.
Safety of trial subjects is monitored by an independent data safety monitoring board (DSMB).

DISCUSSION

The CURSOR trial is designed to determine whether treatment with UDCA may result in a reduction of TLV in symptomatic PLD patients. Furthermore the effect on symptoms and quality of life will be studied. There is a need for medical options that curtail PLD growth and thereby reduces symptoms. A novel therapy should have an excellent safety/efficacy balance while being cost-effective. Current therapies such as liver transplantation are effective but carry a high mortality risk, and in view of shortage of liver-donors is an option that is not readily available. [1] Somatostatin therapy is a promising option, but is expensive, its efficacy is not universal, and side effects after prolonged administration such as hyperglycemia, bradycardia and development of gallstones can be obstacles for widespread use. Alternative options are therefore urgently needed.

Ideally, a drug that is effective in PLD meets with several requirements. The drug should possess a large treatment effect that is able to reverse the natural course of the disease. The effect should not only diminish TLV but also improve a wide spectrum of domains that compose the quality of life. The effect should be quickly visible, predictable and universal. Likewise the drug should be cheap and widely available and come in an oral administration form. Lastly, a finite short therapy course should be enough to result in a long-lasting effect. UDCA meets with some of the requirements and there is a solid biological plausibility that supports its use in PLD.

Cystogenesis in PLD is associated with increased cAMP and decreased calcium levels. [5, 6, 29] Theoretically reversal of raised intracellular cAMP and decreased calcium levels might be an effective approach to reduce polycystic liver volume. UDCA is registered and used in several cholestatic disorders such as PBC and intrahepatic cholestasis during pregnancy. [13, 14, 30, 31] Placebo-controlled trials support the anticholestatic efficacy of UDCA in PBC. Biomarkers such as GGT and ALP markedly decrease in UDCA treated PBC patients. Interestingly, elevations of GGT (51%) and ALP (17%) are frequently observed in severe PLD. [32] Probably elevations of GGT and ALP reflect increased cholangiocyte activity and curtailing or even reversing this process might be beneficial in PLD.

To this end we designed a clinical trial that is powered to detect a significant change in polycystic liver volume in PLD patients treated with UDCA. In addition we will evaluate symptoms and HRQL after 24 weeks of therapy, as well as changes in biomarkers such as GGT and ALP. Finally, we aim to study safety and tolerability of UDCA.

The strength of this trial is that we will include patients with a more severe phenotype such as those with symptoms and grossly enlarged livers (threshold ≥ 2500mL). This is the patient population that is in need of therapy as HRQL is reduced in these patients. [2] Furthermore the international character of this trial makes it possible to generalize findings to a larger population of patients. If UDCA treatment would lead to a >4% difference in change in TLV relative compared to no treatment this would suggest UDCA to be equal or even superior to the effect of somatostatin analogues.

There are some limitations that are worth mentioning. First, it would be most desirable to have a formal placebo controlled design to include placebo effects, especially when assessing symptom reduction and HRQL improvement. However, our primary aim (reduction in TLV) is an objective measure that cannot be influenced by patients, hence the reason to obviate placebo. Secondly, ADPKD patients with MDRD <30 ml/min/1.73m^2 will be excluded from the trial, as UDCA has never been studied in this patient group. Therefore the results of this study cannot be generalized to this specific subgroup of ADPKD patients with symptomatic PLD.

In conclusion, by designing the CURSOR trial we hypothesize that UDCA therapy in symptomatic PLD patients reduces TLV, reduces symptoms and improves HRQL.

Intentionally we have chosen to first explore effect of UDCA monotherapy, in order to prevent influence of other therapies. Combining UDCA with for example somatostatin analogues could hypothetically result in a synergistic effect. This should, as well as dose-response relationship and the molecular mechanism of action of UDCA in PLD be studied in a next trial.

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Chapter 6b

Ursodeoxycholic acid in advanced polycystic liver disease: A phase 2 multicenter randomized controlled trial

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ABSTRACT

Background and aims

Ursodeoxycholic acid (UDCA) inhibits proliferation of polycystic human cholangiocytes in vitro and hepatic cystogenesis in a rat model of polycystic liver disease (PLD) in vivo. Our aim was to test whether UDCA may beneficially affect liver volume in patients with advanced PLD.

Methods

We conducted an international, multicenter, randomized controlled trial in symptomatic PLD patients from three tertiary referral centers. Patients with PLD and total liver volume (TLV) ≥ 2500 ml were randomly assigned to UDCA treatment (15-20mg/kg/day) for 24 weeks, or to no treatment. Primary endpoint was proportional change in TLV. Secondary endpoints were change in symptoms and health-related quality of life. We performed a post-hoc analysis of the effect of UDCA on liver cyst volume (LCV).

Results

We included 34 patients and were able to assess primary endpoint in 32 patients, 16 with autosomal dominant polycystic kidney disease (ADPKD) and 16 with autosomal dominant polycystic liver disease (ADPLD). Proportional TLV increased by 4.6 ± 7.7% (mean TLV increased from 6697 ml to 6954 ml) after 24 weeks of UDCA treatment compared to 3.1 ± 3.8% (mean TLV increased from 5512 ml to 5724 ml) in the control group (p=0.493). LCV was not different after 24 weeks between controls and UDCA treated patients (p=0.848). However, UDCA inhibited LCV growth in ADPKD patients compared to ADPKD controls (p=0.049).

Conclusions

UDCA administration for 24 weeks did not reduce TLV in advanced PLD, but UDCA reduced LCV growth in ADPKD patients. Future studies might explore whether ADPKD and ADPLD patients respond differently to UDCA treatment.

INTRODUCTION

Polycystic liver diseases (PLDs) are genetic disorders that lead to the formation of cysts throughout the liver. [1] PLD is present in a large proportion of patients with autosomal dominant polycystic kidney disease (ADPKD), a disorder where the majority of patients (94%) develop hepatic cysts in addition to kidney cysts. [2] Multiple hepatic cysts can also appear in patients without renal involvement (i.e., autosomal dominant polycystic liver disease (ADPLD)). Due to progressive cyst growth, patients can develop hepatomegaly. This could lead to symptoms such as abdominal pain, early satiety and an impaired health-related quality of life (HRQL). [1, 3, 4] Current therapies for PLD such as fenestration and liver transplantation are invasive with high risk of complications. [5] Medical treatment with somatostatin analogues does hold some promise and is able to reach a total liver volume (TLV) reduction of ~5% in 6-12 months. [6-8] However, not all patients do respond and some may develop side effects such as glucose intolerance, diarrhea or gallstones. Moreover, somatostatin analogues are very expensive. Therefore, other options are needed.

The genetic profile of ADPKD and ADPLD is distinct but the resulting liver phenotype is similar.[1] ADPKD is mainly caused by mutations in the polycystic kidney disease 1 gene (PKD1) or PKD2 gene, while ~25% of ADPLD cases have a mutation in one of the three known genes PRKCSH, SEC63 or LRP5. [9] The PKD genes encode for polycystin 1 and 2 respectively, both integral membrane proteins acting as a Ca2+ permeable receptor channel complex. [10] Mutations in polycystins result in decreased intracellular calcium levels (Ca2+i) and subsequent increased intracellular cyclic adenosine monophosphate (cAMP) levels [10, 11]. This promotes the hyperproliferation of cystic cholangiocytes and is a crucial step in hepatic cyst formation that might serve as a potential target for novel pharmacological therapy. [10-13] In this regard, previous studies have shown that cholangiocytes from PCK rats, an animal model with PLD resembling human PLD, have increased intracellular cAMP levels and diminished Ca2+I levels compared to normal human cholangiocytes. Experimental restoration of the Ca2+I levels with a calcium ionophore inhibited cAMP-mediated hyperproliferation of PCK rat cholangiocytes. [11] Thus, strategies aimed to normalize the reduced Ca2+i levels in polycystic cholangiocytes are considered of potential therapeutic value. [10]

The hydrophilic bile acid, ursodeoxycholic acid (UDCA), is a well-known Ca2+ agonist in hepatocytes [14] and cholangiocytes [15]. We recently demonstrated that UDCA restores diminished Ca2+I levels in polycystic human cholangiocytes in culture and decreases hepatic cystogenesis in PCK rats after 5 months of treatment. [16, 17] This beneficial effect of UDCA was also associated with downregulation of the high concentration of cytotoxic bile acids found in PCK rat livers. [17] UDCA is safe and well tolerated in the treatment of patients with primary biliary cholangitis and gallstone disease. [18]

We hypothesized that 6 months of UDCA treatment leads to reduction in liver volume, symptoms and improvement of HRQL in PLD. Therefore, we designed an international, multicenter, randomized controlled phase 2 trial with proportional change in TLV as the primary endpoint.

MATERIAL AND METHODS

Study population

We included symptomatic PLD patients between 18 and 80 years with an underlying diagnosis of ADPLD or ADPKD, and a TLV ≥ 2500 ml. PLD was defined as the presence of ≥ 20 liver cysts on computed tomography (CT) or magnetic resonance imaging (MRI) scan, and ADPKD diagnosis was based upon modified Ravine criteria. [19] Liver volume was judged by one of the investigators and based on clinical findings (symptoms and physical examination), imaging or former TLV assessments. Symptomatic PLD was defined as an Eastern cooperative oncology group – performance status of ≥ 1 and the appearance of at least three of the following symptoms: abdominal pain, abdominal distension, abdominal fullness, dyspnea, early satiety, back pain, nausea/vomiting, anorexia, weight loss and jaundice. [20] Full details of inclusion and exclusion criteria are shown in Supplementary material and methods.

This trial was conducted at three university centers specialized in PLD: one in Spain (Donostia University Hospital, San Sebastián, Spain) and two in the Netherlands (Academic Medical Center Amsterdam and Radboud university medical center, Nijmegen).

Trial design and treatment allocation

Eligible patients were randomly assigned in blocks of four in a 1:1 ratio to receive UDCA (Ursochol, Zambon, the Netherlands), orally twice a day, in a dose of 15-20mg/kg/day for 24 weeks, or to undergo follow-up without any clinical trial treatment. Sequence generation was handled by an independent researcher using www.randomization.com. To ensure allocation concealment, all randomization numbers were placed in opaque, sealed envelopes bundled per four. Envelopes were opened by an independent researcher one day before baseline in order to prepare medication. The independent researcher passed details of group allocation on to the clinical researcher of each center.

UDCA was provided by the local pharmacy of every center. Treatment was initiated the day after baseline visit. Compliance with medication was assessed at week 24 by pill count. During the trial, patients were not allowed to undergo interventions such as aspiration sclerotherapy or surgery, or to use somatostatin analogues.

Study procedures

A 36-week follow-up period was planned, in which a total of five visits at the outpatient clinic were scheduled: week 0 (baseline), 4, 12, 24 (end of treatment) and 36 (follow-up) (Fig. 1).

For safety measures, aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), bilirubin (direct and total), gamma-glumatyltransferase (GGT), alkaline phosphatase (AP), creatinine and international normalized ratio (screening only) were assessed during all visits and adverse events were recorded. At week 0 and 24 CT scans without contrast were performed on a multidetector CT scanner . CT scans had a slice thickness of 3 mm.

For analysis of the primary outcome, all CT scans were blinded to patient identity, treatment allocation and date of scan. Scans were measured in random order. TLV and total kidney volume (TKV) were calculated by 3D measurement of CT scan slices using Pinnacle3® version 9.6 g (Philips Healthcare in Fitchburg, WI, USA). [21] Liver and kidneys were outlined manually every 9 mm. Software interpolated intermediate slices and calculated areas within the indicated circumference, and finally, TLV and TKV were determined. To test whether TLV measurements were reliable, a random set of 18 CT scans (9 baseline and 9 week 24) were measured by two researchers (HD & MN) and inter-observer variation was assessed using a Bland-Altman plot. Bland-Altman plot showed a mean difference of -0.2 ± 2% between the two researchers. TLVs from one researcher (HD) were used for analysis of primary outcome.

Liver cyst volume (LCV) was measured blindly, by fully automatic segmentation of liver images using an image processing pipeline built in MeVisLab (version 2.7.1, MeVis Medical Solutions AG, Bremen, Germany) inspired by Ruggenenti. [22] Parameters for automatic segmentation were maintained constant for all patients to prevent variability between measurements. Images were initially smoothed by an anisotropic diffusion filter, using the modified curvature diffusion equation (time step 0.0625, conductance parameter 3, number of iterations 15). [23] This filter reduces image noise without compromising edges or other important details in the image. Subsequently, images were marked with the TLV segmentation exported from Pinnacle (border voxelized at midpoint, in order to reproduce pinnacle TLV values), and Otsu thresholding (512

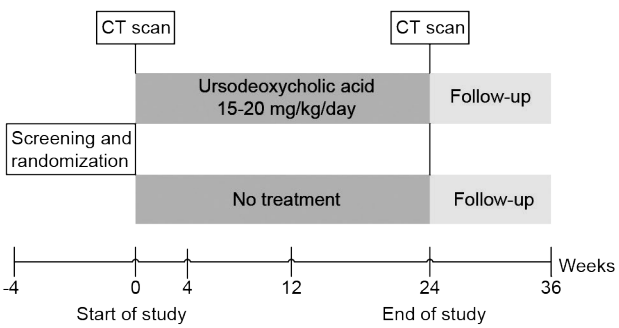

Figure 1. Trial design of the CURSOR trial. Patients were screened for eligibility and eligible patients were randomized in an equal ratio to either the UDCA group or the control group. All patients received a CT scan at baseline and 24 weeks. Control visits were performed at week 4, 12 and 24 after baseline. A follow-up visit was performed 12 weeks after end of study (week 36).

bins) was performed to divide the liver into two classes [24]: cystic volume and parenchyma, based on the image histogram. TLV and LCV were calculated from these segmentations.

Endpoints

Primary outcome of this trial was proportional change in TLV from baseline to week 24 between UDCA group and control group. Secondary endpoints were: change from baseline to 24 weeks in (i) absolute and height-adjusted TLV (hTLV), (ii) absolute and height-adjusted total kidney volume (hTKV), (iii) symptoms, and (iv) HRQL. In addition, safety and tolerability were evaluated. Analysis of LCV as a secondary outcome parameter was added to the protocol after the trial had started in order to relate our findings to the results in PCK rats treated with UDCA. [17]

Symptoms were assessed using the PLD questionnaire (PLD-Q) and gastrointestinal-questionnaire (GI-Q). The PLD-Q is a recently developed and validated questionnaire for PLD patients that includes 13 items about frequency and discomfort of PLD-specific symptoms such as early satiety and abdominal pain. [25] The GI-Q includes 11 items related to abdominal symptoms. [26, 27] Generic HRQL was measured by the medical outcomes study 36-Item short-form health survey (SF-36) and the European organization for research and treatment of cancer quality of life questionnaire core-30 (EORTC). The SF-36 consists of eight scales resulting in a norm-based summarizing physical (PCS) and mental component score (MCS). The EORTC is a validated questionnaire that includes nine symptom scales. Finally, we measured overall HRQL using the visual-analogue scale score of the European quality of life-5 dimension (VAS-EQ5D). Scoring manuals were used to calculate scores and to handle missing items.

Sample size and statistical analysis

A change in TLV of 4% in favor of UDCA compared to no treatment was assumed to be clinically relevant, based on previous trials with somatostatin analogues. [28] A priori sample size calculation revealed a sample size of minimum 34 patients for a statistical power of 80%, a type I error of 0.05 using a two-tailed test, a standard deviation of 4% and a dropout rate of 10%. Clinical outcome variables were analysed on a modified intention-to-treat basis defined as all randomly assigned patients. No interim analyses were done.

Continuous variables were expressed as mean (95% confidence interval (CI)) if normally distributed, otherwise as median (interquartile range (IQR)). Primary outcome and secondary outcomes on TLV, TKV, HRQL and symptoms, were tested with independent t-tests between groups and paired sampled t-tests comparing baseline and end of study within groups. There were no methods used to correct for missing outcomes in the analyses of primary and secondary endpoints. Adverse and serious adverse events were counted per group and patient. Most frequent adverse events and all serious adverse events were reported. A chi-squared test was used to compare numbers of episodes of adverse events between the control and UDCA group. In order to assess differences in response to UDCA, post-hoc subgroup analyses of ADPKD and ADPLD patients' outcomes were performed for primary and secondary outcomes.

All p values calculated were two-tailed, and a p value< 0.05 was considered statistically significant. Analyses were performed using SPSS 22.0 (SPSS Statistics, Inc., Chicago, IL, U.S.A).

Ethical consideration and registration

Ethical approval for the two Dutch centers was obtained from the local institutional review board, i.e. the committee human research region Arnhem-Nijmegen (CMO Arnhem-Nijmegen). For the Spanish center, ethical approval was obtained from the ethics committee for clinical research (CEIC-Euskadi). The study was performed in accordance with the guidelines of Good Clinical Practice/ ICH and the principles of the Declaration of Helsinki. Every patient signed informed consent. Safety of trial subjects was monitored by an independent data safety monitoring board. This trial is registered at https://www.clinicaltrialsregister.eu/, EudraCT Number: 2013-003207-19, and at https://www.clinicaltrials.gov/, identifier: NCT02021110.

RESULTS

Study population

From May 2014 through February 2015, 38 patients were screened for eligibility and 34 patients were randomized. A flow chart of the study population is shown in Fig. 2. All patients completed the total follow-up of 36 weeks by November 2015. Imaging analysis revealed that one patient (UDCA group) did not meet the inclusion criterion TLV ≥ 2500 mL; this patients was excluded from further analyses. Another patient was excluded from analysis of primary outcome only, as baseline CT scan was missing (UDCA group). In total 32 patients were analyzed for primary outcome and 33 for secondary outcomes. Median age was 53 years [IQR: 42-58 years] in the UDCA group and 48 years [IQR: 43-53 years] in the control group (Table 1). In the control group 7 patients (40%) had ADPKD, compared to 9 patients (60%) in the UDCA group. Mean hTLV was 3207 ml/m (95% CI: 2627-3786 ml/m) and 3940 ml/m (95% CI: 2722-5157) ml/m in the control and UDCA group, respectively.

Mean dose of UDCA in the intervention group was 19.9 ± 0.7 mg/kg/day. Compliance, assessed by the average number of pills taken, was 97.0 ± 3.0%. There were no dose reductions or drug discontinuations during the trial.

Liver volume

The proportional change in TLV from baseline to 24 weeks between both arms was not significantly different (UDCA group: 4.6% vs. control group: 3.1%, p = 0.493) (Fig. 3). Mean TLV increased from 6697 ml (95% CI: 4605-8788 ml) at baseline to 6954 ml (95% CI: 4781-9127 ml) at week 24 in the UDCA group, indicating a mean relative increase of 4.6% (95% CI: 0.3%-8.8%) (Table 2). TLV in the control group increased from 5512 mL (95% CI: 4445-6579 mL) to 5724 mL (95% CI: 4548-6900 mL), a mean relative increase of 3.1% (95% CI: 1.1%-5.1%). Individual changes in TLV for both groups showed that TLV decreased in 3 patients treated with

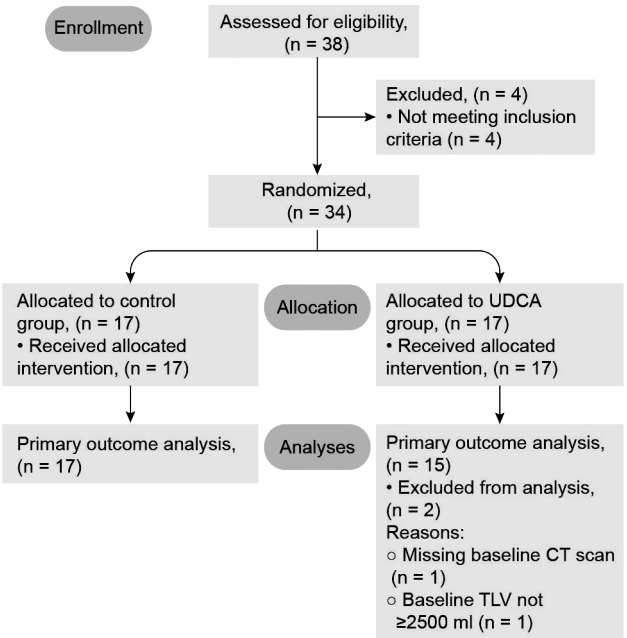

Figure 2. Flow chart of the CURSOR trial. Of the 38 patients assessed for eligibility, 34 were found eligible and were included in the trial. A total of 17 patients were assigned to UDCA and 17 patients to no treatment. Two patients were excluded from analysis of the primary outcome, both randomized to the UDCA group.

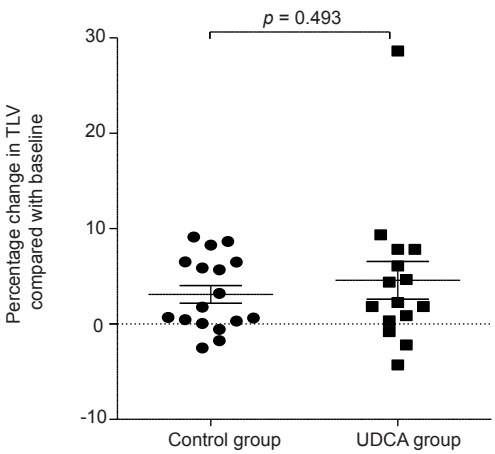

Figure 3. Percentage change in TLV after 24 weeks. TLV increased with 3.1% in the control group vs. 4.6% in the UDCA group. This change was not significantly different (p = 0.493).

Table 1. Baseline demographics and clinical characteristics

	Control group (n=17)	UDCA group (n=15)
Demographics		
Age (years)[a]	48 [43;53]	53 [42;58]
Sex (female)[a]	16 (94%)	12 (80%)
Diagnosis		
ADPKD	7 (41%)	9 (60%)
ADPLD	10 (59%)	6 (40%)
Age at diagnosis[a]	38 [34;42]	43 [36;50]
Years of diagnosis	11 (8;14)	9 (6;12)
Vital statistics		
Weight (kg)	78 (72;85)	81 (74;88)
BMI (kg/m^2) [a]	27 [25;29]	28 [26;30]
Imaging		
TLV (mL)	5512 (4445;6579)	6697 (4605;8788)
hTLV (mL/m)	3207 (2627;3786)	3940 (2722;5157)
TKV (mL)[b]	1543 (319;2768)	1545 (389;2701)
hTKV (mL/m) [b]	897 (189;1605)	904 (240;1567)

Data are reported as median a [IQR], mean (95% CI) or absolute numbers (%). Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ADPLD autosomal dominant polycystic liver disease, BMI, body mass index; hTKV, height adjusted total kidney volume; hTLV, height adjusted total liver volume; TKV, total kidney volume; TLV, total liver volume; UDCA, ursodeoxycholic acid. bADPKD patients only.

UDCA and in 3 patients in the control arm (Fig. 4). One patient (UDCA group), diagnosed with ADPLD, had an extreme increase in TLV of 30%. A sensitivity analysis of primary endpoint in which this patient was excluded, did not change results. There was no significant change in proportional TLV from baseline to week 24 between UDCA and control group in a subgroup analysis of ADPKD and ADPLD patients (respectively p=0.267 and p=0.210).

In addition, there was no statistically significant difference in hTLV after 24 weeks between UDCA group (152 ml/m, 95% CI:32-272 ml/m) and control group (121 ml/m 95% CI:41-201 ml/m) (p = 0.642). Notably, in a subgroup analysis of ADPKD patients, hTLV significantly increased in the control group (172 ml/m, 95% CI:54-302, p = 0.018) compared to a non-significant increase in the UDCA group (152 ml/m, 95% CI:-16 -319, p = 0.071) this increase was not statistically different between both groups (p = 0.835). In ADPLD patients, hTLV did not change within and between UDCA and control group respectively (85 ml/m, 95% CI:-31-202 ml/m vs. 153 ml/m, 95% CI -92-398 ml/m, p = 0.507).

Table 2. Primary and secondary volumetry outcomes

		Control group (n=17)				UDCA group (n=15)				
	Diagnosis	Baseline	Week 24	Change Absolute (mL), Proportional (%)	p-value[a]	Baseline	Week 24	Change Absolute (mL), Proportional (%)	p-value[a]	p-value[b]
TLV (mL)	Both	5512 (4445;6579)	5724 (4548;6900)	212 (70;354) 3.1 (1.1;5.1)	0.006**	6697 (4605;8788)	6954 (4781;9127)	258 (57;458) 4.6 (0.3;8.8)	0.015*	0.689 0.493
	ADPKD	6548 (4524;8571)	6845 (4674;9016)	297 (63;531) 4.3 (1.3;7.2)	0.021*	7422 (4155;10688)	7675 (4171;11179)	254 (-21;529) 2.6(0.5;4.6)	0.066	0.789 0.267
	ADPLD	4787 (3539;6035)	4939 (3516;6363)	152 (-55;359) 2.3(-0.7;5.3)	0.131	5609 (2516;8702)	5872 (2992;8753)	264 (-158 ;685) 7.6%(-4.7;19.8)	0.169	0.528 0.210
hTLV (mL/m)	Both	3207 (2627;3786)	3327 (2689;3966)	121 (41;201)	0.006**	3940 (2722;5157)	4092 (2820;5363)	152 (32;272)	0.017*	0.642
	ADPKD	3806 (2704;4908)	3978 (2798;5158)	172 (54;302)	0.018*	4398 (2492;6304)	4550 (2497;6603)	152 (-16;319)	0.071	0.835
	ADPLD	2787 (2133;3441)	2872 (2122;3622)	85 (-31;202)	0.132	3252 (1510;4993)	3404 (1779;5030)	153 (-92 ;398)	0.170	0.507
LCV (mL)	Both	3346 (2616;4076)	3722 (2812;4631)	376 (131;620)	0.005**	4427 (2667;6188)	4770 (2936;6603)	342 (63;621)	0.020*	0.848
	ADPKD	3774 (2794;4755)	4245 (3007;5482)	470 (100;840)	0.018*	6081 (1122;11040)	6161 (1219;11104)	81 (-103;264)	0.289	0.049*
	ADPLD	2560 (1489;3631)	2762 (1471;4055)	202 (-56;461)	0.100	3601 (1798;5403)	4074 (2000;6147)	473 (63;882)	0.028*	0.296
TKV (ml)	ADPKD	1543 (319;2768)	1578 (335;2822)	35 (1.5;68.7) 0.5 (-0.0;1.0)	0.043*	1545 (389;2701)	1560 (406;2715)	15(-12;43) 0.6(-0.4;1.5)	0.230	0.294 0.858
hTKV (mL/m)	ADPKD	897 (189;1605)	917 (199;1635)	20 (0.7;39.5)	0.044*	904 (240;1567)	913 (250;1577)	10 (-6.7;25.7)	0.213	0.335

Data are reported as mean (95% CI).

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ADPLD autosomal dominant polycystic liver disease; hTKV, height adjusted total kidney volume; hTLV, height adjusted total liver volume; LCV, liver cyst volume; TKV, total kidney volume; TLV, total liver volume; UDCA, ursodeoxycholic acid.

[a] comparison within groups (paired analyses), [b] comparison between groups (unpaired analyses). * $p < 0.05$, ** $p < 0.01$.

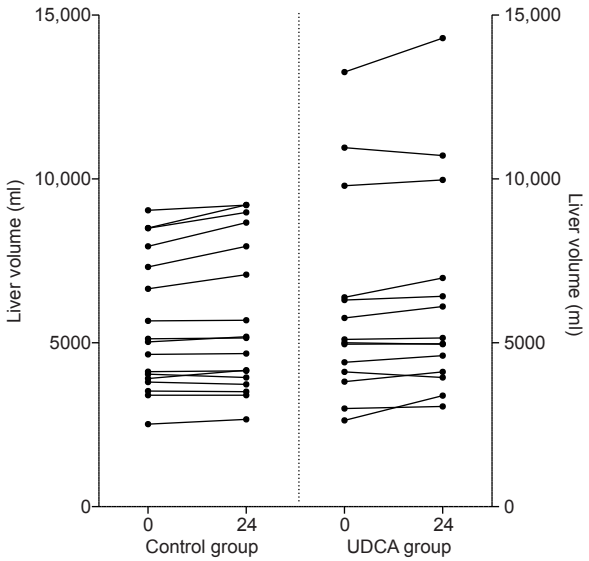

Figure 4. Individual TLV changes in the control and UDCA group after 24 weeks. A total of 28 patients show an increase in TLV, while TLV decreases in 6 patients, 3 in the control and 3 in the UDCA group.

Liver cyst volume

Mean LCV increased 376 ml (95% CI: 131-620 ml) in the control group compared to 342 ml (95% CI: 63-621 ml) in the UDCA group (p = 0.848) (Table 2). Notably, sub-group analysis in ADPKD patients disclosed a significantly higher increase in LCV in the control group (470 ml, 95%CI: 100;840 ml) compared to the UDCA group (81 ml, 95%CI: -103;264 ml) (p = 0.049). In contrast, in ADPLD patients there were no differences in LCV change between the UDCA and control group detected (473 ml, 95%CI: 63;882ml vs. 202 ml, 95% CI:-56;461ml, p = 0.296).
Kidney volume

Proportional change in TKV of ADPKD patients (n=16) from baseline to week 24 was not different between the UDCA and control group (0.5% vs. 0.6%, p = 0.858). Interestingly, hTKV increased significantly from 897 ml/m (95% CI: 189-1605) to 917 ml/m (95%CI: 199-1635) in the control group (p = 0.044) but not in the UDCA group (904 ml/m to 913 ml/m, p = 0.213). Though, analysis between groups showed no statistical significant change (p=0.335) (Table 2).

Symptoms and quality of life

EORTC score improved by 6 points in UDCA treated patients and worsened by 4 points in control group patients (p=0.039) (Supplementary Table 1). In a subgroup analysis of UDCA treated ADPLD patients, EORTC score improved by a mean decrease of 10 points (95% CI: -20;0, p = 0.047) while score increased with 2 points in the control group (95% CI: -7;11, p = 0.628). This improvement in the UDCA group tended to be larger than in the control group (p = 0.064).

No significant symptom improvement was seen in PLD-Q and GI-Q symptom scores (respectively, -3 vs. -7 p = 0.306 and -0.1 vs -0.3, p = 0.419). Quality of life as measured by PCS and MCS score of SF-36 and VAS-EQ5D were not different from baseline to week 24 between control and UDCA group (respectively, p = 0.505, p = 0.819 and p = 0.255).

Safety endpoints: serum liver tests

No changes in biochemical tests were observed from baseline to week 24 between treatment arms, except for GGT (Supplementary Table 2). GGT significantly decreased in the UDCA group from 2.45 times upper limit of normal (ULN) (IQR: 1.18-4.71 times ULN) to 0.75 times ULN (IQR: 0.49-1.00 times ULN) and increased in the control group from 1.58 times ULN (IQR: 1.00-3.15 times ULN) to 1.85 (IQR: 0.97-3.49 times ULN) times ULN (p<0.001 between treatment groups). In addition, AP decreased in the UDCA group (p = 0.017) but not in the control group (p = 0.277). Though, change in AP was not statistically different between groups (p = 0.086).

Adverse events

Three patients were hospitalized during the trial: one patient (UDCA group) because of a brain contusion after falling down the stairs, one patient (control group) suffered from severe abdominal pain suspected for a liver or kidney cyst rupture, and one patient (control group) because of a shoulder injury. In addition, one patient (control group) was diagnosed with breast cancer during the trial. There were no serious adverse events related to the study drug.

A total of 15 (94%) participants in the UDCA group and 12 (71%) in the control group had at least one adverse event (p=0.085) (Supplementary Table 3). Most common adverse events in the UDCA group compared to the control group were frequent stools or diarrhea (38% vs. 12%, p = 0.017) probably related to the study drug.

DISCUSSION

The objective of this study was to evaluate the efficacy and safety of UDCA in patients with advanced PLD with an underlying disease of ADPKD or ADPLD. Our results indicate that UDCA treatment for 24 weeks did not reduce TLV in patients with advanced PLD. Proportional liver volume, hTLV and absolute liver volume were unaffected by UDCA in the whole treatment group and remained within margins seen in controls. However, post-hoc analysis revealed beneficial effect of UDCA on LCV growth in ADPKD compared to ADPLD. Therefore, the effect of UDCA on liver disease in ADPKD need further exploration.

Our main findings of the effect of UDCA on TLV in PLD are in line with results from an uncontrolled pilot study that reported on a 1-year UDCA treatment of 7 PLD patients. [29] The results of this study showed no statistically significant difference between liver growth one year before treatment and one year after treatment, but indicated a tendency of liver growth

inhibition in the UDCA group. However, results need to be interpreted with caution as the sample size was small, no control group was included, and a very low dose of UDCA (300mg/day) was applied. [29]

The main question that needs to be discussed is why UDCA failed to reduce TLV in our study population. Our hypothesis that UDCA reduces TLV in advanced PLD was based on experiments in PCK rats, an animal model of PLD [11, 17], and on former studies on signaling properties of UDCA conjugates in hepatocytes and cholangiocytes [30]. It might be that PCK rats do not recapitulate the whole spectrum of molecular events leading to PLD in humans and that, at best, experimental observations from PCK rats can only be translated to the molecular pathophysiology of some PLD subgroups. Thus, it remains unclear whether the PLD patient population selected for this trial was the adequate target population for UDCA treatment in PLD.

Secondly, it can be debated whether PLD stage in our study population can be compared to that of the PLD stage studied in PCK rats. PCK rats received UDCA for 5 months starting at an age of 8 weeks, when the disease is mild and in progression. [17, 31] In contrast, UDCA therapy was here initiated in patients with advanced PLD and who were diagnosed with PLD for a mean of 11 ± 6 years. In addition, PCK rats have a life span of 1.5 years and received UDCA for 5 months while our study population received UDCA for 6 months on a much longer life span. One could speculate that earlier and more sustained intervention with UDCA might be more effective than a short-term intervention at an advanced stage of PLD.[17]

A third explanation might be that the effect of UDCA is smaller than the effect size we powered on. The a priori calculated number of patients needed for our study was based on the power to detect a clinical difference of at least 4% of TLV over 6 months, but not LCV as tested in PCK rats. This effect size was based on former studies with somatostatin analogues. [28] It is possible that UDCA affects liver volume in PLD, but the short-term effect would be smaller than that seen with a 6 month-course of somatostatin analogues. [6-8] In addition, it remains unclear whether longer UDCA treatment (2-4 years) in ADPKD could be more effective than long-term somatostatin treatment considering that LCV was reduced in ADPKD after 6 months in our study.

Interestingly, our results showed a significant improvement in HRQL after UDCA treatment, as measured by EORTC questionnaire, while scores on other HRQL symptom questionnaires remained unchanged. As change in TLV after 24 weeks of UDCA treatment did not differ compared to change of TLV in the control group, chance or a placebo effect might be the root cause for the improvement in HRQL.

This brings us to the first limitation of our trial: the lack of double-blinding for treatment allocation. However, the primary outcome change in TLV, was analyzed in a blinded objective fashion. Therefore, we assume that the absence of blind patients and physicians did not affect

our primary outcome. However, it could affect secondary outcomes such as HRQL and symptom burden. Secondly, our study was not powered for subgroup analyses of ADPKD and ADPLD patients. Thus, subgroup analyses were explorative by nature. The positive effects of UDCA treatment on LCV in the subgroup of patients with ADPKD, although borderline significant, are intriguing and might be studied in the future.

The international multicenter design of our trial was our key strength as it increases the generalizability of our findings. Another absolute strength of our trial is that we included a control group and were able to compare the effect of UDCA to standard of care.

In conclusion, UDCA administration showed no benefit in reducing TLV in advanced symptomatic PLD patients but decreased LCV in ADPKD patients. Further exploration of differences between ADPKD and ADPLD patients in the treatment response to UDCA, minimum duration and dose of UDCA treatment, appear warranted. Future studies should also focus on unraveling additional molecular targets involved in cystogenesis of different forms of PLD.

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SUPPLEMENTARY FILES

Supplementary File 1. Inclusion and exclusion criteria

Inclusion criteria

- 18 ≤ age ≤ 80 years
- PLD, defined as ≥ 20 liver cysts on CT or MRI scan, with underlying diagnosis of ADPLD or ADPKD
- TLV ≥ 2500 mL
- Symptomatic defined as ECOG ≥ 1 (31), and having at least three out of ten PLD symptoms
- Signed informed consent

Exclusion criteria

The following exclusion criteria will be assessed by questioning the patient and assessing patient his medical file.

- Use of oral contraceptives or estrogen supplementation
- Use of UDCA within three months prior to baseline
- Females who are pregnant or breast-feeding or patients of reproductive potential not employing an effective method of birth control.
- Intervention (aspiration or surgical intervention) within six months before baseline
- Treatment with somatostatin analogues within six months before baseline
- Renal dysfunction (estimated glomerular filtration rate calculated by the
- Modification of Diet in Renal Diseases (MDRD) < 30 ml/min/1.73m^2)
- Patients with a kidney transplant
- Hypersensitivity reaction to UDCA or patients with galactose-intolerance, lactase deficiency or glucose-galactose malabsorption
- Acute cholecystitis or frequent biliary colic attacks
- Acute stomach or duodenal ulcers
- Inflammation of small intestine or colon
- Use of drugs that can interact with UDCA, such as colestyramine, aluminium hydroxide or cyclosporine
- Enrolment in another clinical trial of an investigational agent while participating in this study

The following exclusion criteria will be judged by the investigator screening the patient for eligibility:

- History or other evidence of severe illness or any other conditions which would make the patient, in the opinion of the investigator, unsuitable for the study
- Mental illness that interferes with the patient ability to comply with the protocol

Supplementary Table 1. Health related quality of life measures

	Control group (n =17)				UDCA group (n=16)				
	Baseline	Week 24	Absolute change	p-value[a]	Baseline	Week 24	Absolute change	p-value[a]	p-value[b]
PLD-Q score	48 (42;54)[b]	47 (40;54)	-3 (-7;2)	0.088	44 (35;53)	38 (29;46)	-7 (-14;0)	0.060	0.306
ADPKD	45 (34;57)	52 (35;67)	0.8 (-10;12)	0.994	43 (31;54)	36 (25;48)	-6 (-17;- 4)	0.180	0.302
ADPLD	49 (41;57)	45 (37;52)	-5 (-10; -1)	0.010	47 (28;65)	40 (23;56)	-7 (-19;5)	0.240	0.629
GI-Q	2.7 (2.5;3.0)	2.6 (2.2;3.1)	-0.1 (-0.5;0.3)	0.642	2.5 (2.2;2.9)	2.2 (1.9;2.6)	-0.3 (-0.6;0.0)	0.074	0.419
ADPKD	2.7 (2.1;3.3)	2.7 (2.0;3.4)	0.03 (-0.5;0.5)	0.905	2.4 (1.9;2.9)	2.2 (1.6;2.7)	-0.2 (-0.8;0.3)	0.351	0.439
ADPLD	2.7 (2.4;3.1)	2.6 (1.8;3.3)	-0.2 (-0.8-0.5)	0.573	2.7 (2.0;3.3)	2.3 (1.8;2.8)	-0.4 (-0.8;0.1)	0.088	0.631
Physical component score SF-36	40 (35;45)	39 (35;44)	0 (-7;6)	0.582	39 (35;45)	43 (38;49)	3 (-5;11)	0.040	0.505
ADPKD	37 (29;46)	35 (26;43)	-2 (-11;8)	0.332	38 (30;47)	41 (32;50)	3 (-9;15)	0.258	0.516
ADPLD	42 (34;50)	43 (37;48)	1 (-10;11)	0.859	42 (34;50)	46 (40;53)	3 (-10;16)	0.064	0.747
Mental component score SF-36	51 (45;57)	49 (44;55)	-2 (-5;2)	0.273	49 (44;54)	48 (42;53)	-1 (-5;3)	0.548	0.819
ADPKD	53 (39;66)	53 (43;63)	0 (-7;7)	0.989	50 (43;56)	49 (41;57)	-1 (-6;4)	0.680	0.788
ADPLD	50 (43;57)	47 (39;55)	-3 (-7;1)	0.148	48 (38;59)	47 (37;57)	-2 (-11;8)	0.690	0.709
EORTC	27 (20;33)	31 (23;39)	4 (-1;10)	0.132	32 (24;41)[a]	26 (19;34)	-6 (-14;3)	0.163	0.039*
ADPKD	25 (13;37)	32 (19;46)	7 (1;14)	0.035*	32 (19;46)	29 (17;42)	-3 (-17;11)	0.649	0.184
ADPLD	28 (19;36)	30 (18;42)	2 (-7; 11)	0.628	33 (17;48)	22 (11;32)	-10 (-20; 0)	0.047	0.064

Supplementary Table 1. Continued

	Control group (n =17)				UDCA group (n=16)				
	Baseline	Week 24	Absolute change	p-value[a]	Baseline	Week 24	Absolute change	p-value[a]	p-value[b]
VAS-EQ5D	64 (57;72)	60 (52;69)	-2 (-7;4)	0.481	61 (50;72)	66 (58;74)	6 (-7;18)	0.350	0.255
ADPKD	62 (44;80)	59 (41-78)	-2 (-11;6)	0.499	66 (52;79)	63 (51;76)	-2 (-17;12)	0.720	0.99
ADPLD	66 (59;73)	61 (51;71)	-1 (-10;7)	0.733	53 (29;78)	71 (59;83)	18 (-7;42)	0.129	0.12

Note. Data are Mean (95% CI). Abbreviations: EORTC, European organization for research and treatment of cancer quality of life questionnaire;GI-Q, gastrointesintal questionnaire;PLD-Q, polycystic liver disease questionnaire;SF-36, MOS 36-item short form health survey; VAS-EQ5D, Visual Analogue Scale score of the Euro Quality of Live five dimensions questionnaire. Score on PLD-Q, EORTC on a range of 0-100 (0, best imaginable; 100, worst imaginable). SF-36 and VAS-EQ5D (0, worst imaginable, 100, best imaginable). [a] n=15, [b] n=16 , * $p < 0.05$.

[a]: comparison within groups (paired analysis), [b]comparison between groups. * $p < 0.05$, ** $p < 0.01$.

Supplementary Table 2. Laboratory results

	Control group (n=17)				UDCA group (n=16)				
	Baseline	**End**	**Absolute change**	**p-value[a]**	**Baseline**	**End**	**Absolute change**	**p-value[a]**	**p-value[b]**
Creatinine	0.67 [0.63;0.82]	0.69 [0.59;0.81]	-0.00 [-0.06;0.05]	0.476	0.74 [0.66;0.86]	0.75 [0.64;0.78]	-0.03 [-0.05; 0.01]	0.812	0.423
ADPKD	0.82 [0.67;0.99]	0.81 [0.71;0.93]	-0.01 [-0.06;0.06]	0.866	0.68 [0.66;1.01]	0.70 [0.63;0.97]	-0.02 [-0.04;0.03]	0.513	0.758
ADPLD	0.66 [0.58;0.67]	0.78 [0.65;0.87]	0.01 [-0.07;0.04]	0.813	0.78 [0.65;0.87]	0.75 [0.68;0.79]	-0.04 [-0.10;.-0.02]	0.028*	0.272
GGT	1.58 [1.00;3.15]	1.85 [0.97;3.49]	0.07 [-0.29;0.24]	0.326	2.45 [1.18;4.71]	0.75 [0.49;1.00]	-1.13 [-3.62; -0.49]	0.001**	<0.001**
ADPKD	2.55 [1.6;5.1]	3.47 [1.85;5.16]	0.18 [0.05;0.50]	0.176	1.62 [1.20;4.91]	0.75 [0.53;1.08]	-1.13 [-3.84; -0.55]	0.008*	0.001**
ADPLD	1.00 [0.74;2.17]	1.13 [0.75;2.41]	0.02 [-0.56;0.20]	0.859	3.29 [0.63;4.52]	0.68 [0.44;1.07]	-1.62 [-3.00; -1.62]	0.028*	0.029*
AP	0.77 [0.68;1.02]	0.80 [0.67;1.22]	-0.04 [-0.10; 0.03]	0.277	1.04 [0.72;2.54]	0.83 [0.65;1.53]	-0.15 [-0.42; 0.05]	0.017*	0.086
ADPKD	0.80 [0.68;1.02]	0.81 [0.68;1.06]	0.008 [-0.10;0.04]	0.866	0.84 [0.74;1.42]	0.77 [0.62;1.21]	-0.05 [-0.13;0.04]	0.051	0.174
ADPLD	0.74 [0.69-1.42]	0.75 [0.65-1.40]	-0.10 [-0.36;0.02]	0.314	2.11 [0.68-2.73]	1.21 [0.63-1.83]	-0.28 [-0.68;0.08]	0.173	0.272
AST	0.76 [0.58;0.97]	0.71 [0.63;0.94]	-0.03 [-0.13;0.07]	0.518	0.69 [0.50;0.77]	0.60 [0.54;0.68]	-0.05 [-0.23;0.03]	0.182	0.495
ADPKD	0.89 [0.58;1.23]	0.74 [0.55;0.97]	-0.14 [-0.16;0.05]	0.128	0.71 [0.50;0.76]	0.54 [0.50;0.64]	-0.02 [-0.18;0.03]	0.341	0.918
ADPLD	0.74 [0.62;0.83]	0.71 [0.67;0.87]	-0.03 [-0.07;0.18]	0.553	0.68 [0.48;0.89]	0.65 [0.58;0.75]	-0.05 [-0.29;0.10]	0.463	0.388
ALT	0.83 [0.60;0.98]	0.77 [0.59;0.96]	-0.03 [-0.20;0.11]	0.495	0.66 [0.57;0.71]	0.60 [0.46;0.66]	-0.06 [-0.27;0.03]	0.093	0.446
ADPKD	1.00 [0.50;1.20]	0.86 [0.50;0.97]	-0.11 [-0.31;0.03]	0.128	0.66 [0.59;0.80]	0.60 [0.54;0.66]	-0.06 [-0.28;0.03]	0.065	0.918
ADPLD	0.77 [0.66;0.90]	0.77 [0.70;0.93]	0.03 [-0.20;0.23]	0.674	0.66 [0.51;0.71]	0.55 [0.37;0.74]	-0.06 [-0.32;0.19]	0.674	0.456
Direct bilirubin	1.00 [1.00;1.00]	1.00 [0.93;1.00]	0.00 [0.00;0.00]	0.969	1.00 [0.79;1.00]	1.00 [0.70;1.00]	0.00 [0.00;0.00]	1.000	0.586
ADPKD	1.00 [0.71;1.20]	1.00 [1.00;1.00]	0.00 [0.00;0.00]	0.655	1.00 [1.00;1.00]	1.00 [1.00;1.00]	0.00 [-0.11;0.00]	0.317	0.837
ADPLD	1.00 [1.00;1.00]	1.00 [1.00;1.00]	0.00 [0.00;0.00]	0.180	0.57 [0.43;1.00]	0.67 [0.43;0.86]	0.00 [0.00;0.11]	0.317	0.283
Indirect bilirubin	0.54 [0.53;0.93]	0.62 [0.41;0.90]	0.00 [-0.12;0.18]	1.000	0.53 [0.41;0.65]	0.53 [0.41;0.59]	0.00 [-0.12;0.12]	0.581	0.902
ADPKD	0.53 [0.53;1.41]	0.62 [0.41;1.24]	0.00 [-0.16;0.19]	1.000	0.53 [0.44;0.82]	0.53 [0.44;0.59]	-0.06 [-0.26;0.09]	0.360	0.456
ADPLD	0.55 [0.53;0.85]	0.62 [0.40;0.81]	-0.06 [-0.15;0.24]	0.779	0.41 [0.36;0.59]	0.53 [0.36;0.70]	0.06 [-0.06 ;0.24]	0.223	0.328

Supplementary Table 2. Continued

	Control group (n=17)				UDCA group (n=16)				
	Baseline	**End**	**Absolute change**	**p-value[a]**	**Baseline**	**End**	**Absolute change**	**p-value[a]**	**p-value[b]**
Albumin	0.78 [0.74;0.84]	0.80 [0.75;0.86]	0.00 [-0.03;0.04]	1.000	0.76 [0.72;0.86]	0.76 [0.72;0.86]	0.00 [-0.03;0.03]	1.000	0.769
ADPKD	0.78 [0.74;0.86]	0.80 [0.72;0.86]	0.00 [-0.06;0.04]	0.891	0.72 [0.71;0.76]	0.74 [0.72;0.76]	0.00 [-0.03;0.04]	0.739	1.000
ADPLD	0.82 [0.75;0.84]	0.82 [0.77;0.86]	0.00 [-0.02;0.04]	0.683	0.84 [0.74;0.93]	0.87 [0.83;0.91]	0.00 [-0.04;0.03]	1.000	0.755

Data are reported as median times upper limit of normal [IQR]. A total of 33 patients is included in this analysis, UDCA group (n=16), control group (n=17).

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ADPLD autosomal dominant polycystic liver disease; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

[a]: comparison within groups (paired analysis), [b] comparison between groups. * $p < 0.05$, ** $p < 0.01$.

Supplementary Table 3. Number of episodes of adverse events

Adverse event	Control group n (%)[a] n=17	UDCA group n (%) [a] n=16	p value
Digestive tract			
Frequent stools/diarrhea	2 (12)	8 (38)[b]	0.017
Sticky tools	0 (0)	3 (19)	0.061
Bloating belly/abdominal tension	2 (12)	3 (19)	0.576
Stomach cramps	0 (0)	2 (13)	0.133
Flatulence	0 (0)	1 (6)	0.295
Other			
Flue	3 (18)	4 (25)	0.606
Suspicion of cyst rupture (liver/kidney)[c]	1 (6)	3 (13)[b]	0.258
Pneumoniae	2 (12)	1 (6)	0.582
Breast carcinoma[c]	1 (6)	0 (0)	0.325
Brain contusion[c]	0 (0)	1 (6)	0.485
Crushed shoulder[c]	1 (6)	0 (0)	0.325
Dose reductions	NA	0 (0)	-
Drug discontinuation	NA	0 (0)	-

[a] Denominator is total of patients in study arm.

Abbreviations: NA, not applicable.

[b] More than 1 episode of adverse event in 1 patient.

[c] Serious adverse events

Chapter 7

General discussion

GENERAL DISCUSSION

The aim of this thesis was threefold,

1. to assess the role of kidney and liver volume in symptom burden in autosomal dominant polycystic kidney disease (ADPKD) patients;
2. to characterise the population of PLD patients who were in need of therapy and explore factors involved in treatment decisions;
3. to test ursodeoxycholic acid (UDCA) in PLD.

Following these aims, our research questions were as follows;

1. Who should we treat and what should we target?
2. Who do we treat?
3. A novel therapy for PLD?

Answers to the research questions and implications

1. *Who should we treat and what should we target?*

Our national cohort study included data on kidney volume, liver volume and symptom burden of 309 ADPKD patients. We demonstrated that combined kidney and liver volume (hTKLV) in ADPKD was associated with the presence and severity of pain and gastrointestinal symptoms, though correlations were weak. Liver volume (hTLV) determines symptoms, in contrast to kidney volume (hTKV) which is not associated with symptom burden.

These results are in line with a large cohort study in ADPKD patients that demonstrated a weak association between liver volume and the domain "physical functioning" of the SF-36. [1] A cohort study in 92 PLD patients, found no association between liver volume and the physical component score of the SF-36. The physical component score summarizes physical quality of life based on several domains. [2] This study selected patients from two trials that included mainly severe PLD patients who were often symptomatic and had large liver volumes. [3, 4] We think that the large range in liver volumes and symptoms in our cohort contributed to a positive relation between liver volume and symptom burden. [2] The difference in results might also be due to the type of questionnaire. We specifically assessed gastrointestinal symptoms, while former studies used the SF-36, a general questionnaire to measure quality of life. This complicates comparison of results. It is my recommendation to systematically assess PLD symptoms in ADPKD patients via a validated questionnaire developed for PLD, the PLD-Q. [5]

The weak association of liver volume with symptoms implies that other factors contribute to symptom burden as well. In our cohort, a history of liver pain, renal pain, urinary tract infection, liver cyst infection, renal cyst infection, macroscopic hematuria and renal surgery were significantly associated with symptom burden. However, these are factors that cannot be targeted in clinical practice and we should examine whether factors such as coping, that can be targeted, play a role in symptom burden.

The association of volumes with symptoms was analyzed in a cross-sectional fashion. Therefore, we were not able to evaluate the impact of reduction of total kidney liver volume on symptom burden. Our data were extracted from the DIPAK-1 study, an ongoing prospective randomized clinical trial assessing the efficacy of lanreotide on renal function decline, kidney volume and liver volume, in a large group of ADPKD patients. Longitudinal results will follow in the near future, but the weak association between hTKLV and symptoms might implicate that reduction in volume will not improve symptom burden. [6]

Our data show that symptoms were more prevalent in women than men. This gender specificity is in line with results from previous studies. [1, 7] The burden of symptoms in women can be explained by larger liver volumes, a well-known phenomenon in women, most likely a consequence of estrogen levels. [8]

The results of our cross-sectional analysis have several implications. First, our results suggest that we need to target the liver in order to affect symptom burden, especially in women who are more at risk to be symptomatic. Secondly, we need to establish whether other factors contribute to symptom burden and can be a target for therapy. Finally, as liver volume plays a major role in presence and severity of pain and gastrointestinal symptoms, imaging of the liver should be part of the initial assessment of all ADPKD patients, as already been recommended in the Kidney Disease: Improving Global Outcomes guidelines. [9] This will help to diagnose PLD in early disease stages and to intervene appropriately.

2. *Who do we treat?*

The results of our cohort study indicate that the liver should be the main target for therapy. Unfortunately, current therapies that reduce liver volume in PLD are invasive and have high complication and recurrence rates [7], and there are no clear guidelines on treatment of PLD. This might explain the results of our international registry that show a large variation in the decision to treat and treatment strategies, among two European tertiary referral centers for PLD. Our results demonstrated that the choice for a specific therapy such as fenestration or aspiration sclerotherapy is center-dependent. Patients who underwent fenestration or aspiration sclerotherapy had comparable patients 'characteristics without differences in phenotype. This could implicate that expertise drives treatment and that evidence supporting the efficacy of therapies is lacking.

Presence of symptoms and every 10 years of PLD diagnosis increased the likelihood to undergo treatment by 40%. Our data should be interpreted with caution as symptom burden was not systematically assessed through a validated questionnaire. Center played a significant role in the choice for either liver transplantation or aspiration sclerotherapy. This underscores that available expertise drives treatment while evidence that singles out the best treatment modality is lacking.

A high number of patients received multiple treatment strategies (14%). Results from a retrospective cohort study including ADPLD patients showed that 56% of the treated population received more than one treatment strategy. [10] Both results might suggest that current therapies are not effective in reducing symptoms, which is at odds with literature on the efficacy of invasive therapies for PLD. [11-14] Recurrence might be another explanation why patients received multiple treatment strategies.

Liver volume was not available in medical records of most cases included in our registry and we were not able to investigate the role of liver volume on treatment decision or strategy. The lack of liver volumes in medical records could indicate that liver volume assessment is not part of clinical routine or does not play a major role in treatment decision. It might also indicate that routine measurement is not possible due to a lack of equipment or time, as volumetry is time-consuming (45-60 minutes per scan).

In conclusion, a higher number of symptoms and every 10 years of PLD diagnosis increases the likelihood to undergo treatment. Center plays a major role in the choice to elect a particular modality. The absence of the systematically collection of data on symptom burden and liver volume made it impossible to assess efficacy of treatment. Therefore we recommend to assess symptom burden by the PLD-Q, [5] and liver volume by volumetry, both pre- and post therapy to evaluate the efficacy of current treatment strategies.

3. *A novel therapy for PLD?*

According to the results of our registry, a large group of patients in tertiary centers receives invasive therapy (35%). Patients are mostly referred to tertiary centers because of symptomatic PLD. Therefore it might be that even a larger group of patients is in need of therapy but does not fulfil the criteria for current strategies, or is unwilling to receive invasive therapy. Clearly there is a need for a non-invasive therapy. Previous studies exploring the mechanism of cystogenesis in PLD demonstrated that cholangiocytes from PCK rats, an animal model that resembles human PLD, have increased intracellular cAMP levels and diminished Ca^{2+} levels compared to normal human cholangiocytes. Restoration of these levels mediated hyperproliferation in cholangiocytes of PCK rats. [15] UDCA restored diminished Ca^{2+} levels and decreased hepatic cystogenesis in PCK rats after five months of treatment. [16, 17] We designed an international, multicenter, randomized controlled phase 2 trial to assess the efficacy of UDCA in advanced PLD patients.

Our results demonstrate that UDCA treatment for 24 weeks does not reduce total liver volume in patients with advanced PLD. These results are in line with another trial that investigated the effect of UDCA on liver volume. [18] This observational trial demonstrated that there was no statistically significant difference between liver growth one year before and after treatment with UDCA. A limitation is that no control group was included and that a very low dose of UDCA was used. The question is why UDCA failed to reduce total liver volume (TLV) in PLD

patients? One of the explanations might be that the data from the PCK rat model cannot be extrapolated (where somatostatin analogues work [3, 19]) to the situation in humans. Another explanation might be the choice of our study population. We tested UDCA in advanced human PLD, while the PCK rats used in the experiments were only eight weeks old. As a results, UDCA might be effective in early stages of the disease instead of advanced disease. Moreover, PCK rats live about 1.5 years and they received UDCA for five months, while our patients received UDCA for six months but have a much longer life span. This would indicate that we should treat patients for a longer period to achieve a similar effect.

In a post-hoc subgroup analysis, we were able to detect a significant smaller liver cyst growth rate in ADPKD patients treated with UDCA, compared to ADPKD control patients. This effect was not seen in ADPLD. However, results should be interpreted with caution as our sample size was not powered on the effect of total cyst volume.

Our results suggest that there is no role for UDCA in the treatment of PLD, at this moment. The results of our post-hoc subgroup analysis suggest that there might be differences in the molecular mechanisms of cystogenesis in ADPKD and ADPLD patients, that need different therapeutic approaches.

Limitations and strengths

In order to study the association of liver and/or kidney volume with symptom burden in ADPKD patients, we used a cross-sectional analysis. The main strength of this cross-sectional analysis was the use of baseline characteristics of a prospective protocolized trial which prevented missing data. The limitation of a cross-sectional analysis is that no conclusions can be drawn about longitudinal effects such as the role of liver growth in symptom burden. For the timing of treatment, this information is important. However, as our data were extracted from an ongoing prospective randomized clinical trial[6], these results will be available in the near future.

Our PLD registry is a robust method to systematically collect data on factors involved in treatment decision in PLD. The international ground had several advantages. First, it facilitated the inclusion of a large group of patients with a rare disease such as PLD. Second, we could study the effect of center on treatment decision. Third, in the future it will help to select patients for clinical trials. The retrospective character of our data analysis hurdles the exploration of the whole spectrum of factors that might be involved in treatment decision. We were dependent on the information in medical records of patients, which were often incomplete. Our international PLD registry will therefore continue as a prospective database including longitudinal data on liver growth and symptom burden.

A randomized controlled trial was the best method to test whether UDCA is an effective therapy for PLD. The rarity of PLD makes it a challenge to achieve the desired inclusion, but the international character of our trial helped. Ideally, a placebo controlled trial is preferable,

though this is very expensive and since TLV is an objective endpoint a placebo group would probably have not changed the results.

Future perspectives

Future research should focus on the following topics.

1. *The effect of liver volume reduction on symptom burden*

Liver volume plays a role in symptom burden in ADPKD patients, while kidney volume does not. The next step is to assess the effect of liver volume reduction on symptom burden. The result of the DIPAK-1 study should therefore be awaited.[6]

2. *Other determinants for symptom burden*

The weak association between liver volume and symptom burden in our cohort of ADPKD patients suggests that other factors play a role as well. Identification of these elements help to design better targeted interventions and to tailor therapy according to the proportional reduction of liver volume.

3. *Defining an endpoint*

Our PLD registry indicates that there is a lack of evidence on efficacy of therapies for PLD. To evaluate efficacy of therapies through a clinical trial we need well defined endpoints. The most desired outcome of an intervention in PLD is reduction of symptoms, though PLD is often associated with non-specific symptoms. A composite endpoint including liver volume and symptom burden, might be desirable. For symptom burden, a validated PLD specific questionnaire such as the PLD-Q can be used. [5] In order to collect data on liver volume, there is a need for a quick and accurate method to measure liver volume independent of time and place and within a reasonable time frame. Combining a PLD-Q score reduction with liver volume or growth reduction, might be an excellent composite endpoint for assessment of efficacy of PLD therapies.

4. *Efficacy of current therapies*

The results of our registry indicated that the choice for aspiration sclerotherapy or fenestration is center-dependent. A randomized trial to compare the efficacy of both therapies should be performed to elucidate the best therapy is. In addition, physicians in clinical practice could evaluate short- and long-term efficacy of current therapies by assessing liver volume and symptom burden pre- and post treatment. This will help to build an evidence base for current therapies.

5. *Design a non-invasive therapy*

There is definitely a need for a non-invasive therapy for PLD. Future studies should focus on testing drugs that alter mechanism involved in cystogenesis, such as the cAMP mediated hyperproliferation of cystic cholangiocytes, in order to change natural course of PLD.

For UDCA, ongoing research is needed to find out whether longer treatment with UDCA or a higher dose might be effective in PLD. As UDCA might have different effects on cyst volume in ADPKD and ADPLD patients, it might be worth considering to assess disease specific differences.

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ENGLISH SUMMARY

Polycystic liver disease (PLD) is characterized by the development of multiple cysts spread throughout the liver. PLD is present in two genetically distinct disorders, as a primary phenotype in autosomal dominant polycystic liver disease (ADPLD) and secondary to renal cysts in autosomal dominant polycystic kidney disease (ADPKD). The natural course of PLD shows a progressive increase in number and size of hepatic cysts. This can result in hepatomegaly. A normal liver weighs about 1.5L. In patients with severe PLD the liver can grow up to 5-10 times its normal size, although liver function remains intact. As a consequence of progressive cyst growth, patients can develop symptoms such as abdominal pain, early satiety and dyspnea.

For patients with symptomatic PLD several treatment options are available. Aspiration sclerotherapy is indicated in patients with a dominant cyst that is clearly responsible for the symptoms. The procedure involves radiological aspiration of cyst fluid and subsequent administration of a sclerosing agent to destruct the cyst wall. Surgical treatment options are fenestration, resection or liver transplantation. Fenestration involves surgical deroofing of cysts and is indicated in patients with multiple superficial large cysts. Resection means that a segment of the liver will be removed. This is indicated in patients who have a phenotype with at least one segment of unaffected liver parenchyma. Above mentioned therapies are invasive and have a high risk of complications and recurrence. A liver transplantation is the only curative treatment, however this is an invasive procedure and due to scarcity of donors not widely performed in patients with PLD.

Because all available treatment options are invasive, we need to preserve them for those patients that are symptomatic or have a high likelihood to develop symptoms. However, at this moment we are not yet able to predict who will develop symptomatic PLD. Besides, the relation between liver volume and symptom burden is not entirely clear. There are no guidelines to initiate therapy in PLD and we do not know which factors play a role in initiating therapy, and the choice for a specific treatment strategy. Finally, there is a need for a non-invasive therapy that alters natural course in order to reduce or prevent symptoms. This thesis addresses several of these issues.

Former research has shown that there are several risk factors for developing symptomatic PLD. In my thesis, we gave an overview of these risk factors (**chapter 2**).

It seems logical that an increase in liver volume is associated with symptoms. Though, evidence is inconclusive. In this thesis we have studied the relation of combined liver and kidney volume with symptoms (**chapter 3**). We explored this relation in a population of ADPKD patients, as PLD is mainly present in this disorder. Results showed that a combination of kidney and liver volume is associated with symptom burden with a prominent role for liver volume. There was

no association between kidney volume and symptoms. These findings implicate that physicians should target liver volume to reduce symptoms in ADPKD patients.

However, there are no guidelines that help physicians to choose a specific treatment for patients with PLD. To find out which factors play a role in the initiation of therapy and the choice for specific treatment strategies, we created an international registry. First, we explored the literature to find the elements needed for a successful database (**chapter 4**). The results of our international registry showed that 35% of patients from one of both nationwide tertiary centers, received invasive treatment (**chapter 5**). The likelihood to receive treatment increased with number of symptoms and with the number of years the diagnosis of PLD was present. The choice for either liver transplantation or aspiration sclerotherapy was center-dependent. Differences in treatment decisions between both centers implicated that there is a lack of evidence on the best treatment strategy for PLD.

As current therapies are invasive and have high complication risks and recurrence rates, we developed an international trial to assess the efficacy of ursodeoxycholic acid (UDCA) to reduce liver volume in PLD (**chapter 6a**). In total we included 34 patients with symptomatic hepatomegaly. Patient were randomized to receive either UDCA or no treatment for 24 weeks. Results showed that UDCA was not effective in reducing liver volume (**chapter 6b**). A post-hoc subgroup analyses revealed that liver cyst growth was less in UDCA-treated ADPKD patients versus ADPKD patients who received no treatment. This result was not shown in ADPLD patients. At this moment there is no role for UDCA in the treatment of PLD. Future research needs to find out whether ADPKD and ADPLD patients respond differently to UDCA.

The results of my thesis showed that liver volume plays a role in symptomatic ADPKD and that treatment should target the liver in order to reduce symptom burden. Current therapies that target liver volume however, are very invasive and center seemed to play an important role in the treatment decision and the strategy chosen. Therefore, future studies are needed to investigate the efficacy of current therapies. UDCA as a non-invasive therapy for PLD has no role in the treatment of symptomatic PLD at this moment.

NEDERLANDSE SAMENVATTING

Polycysteuze leverziekte (PLD) kenmerkt zich door de ontwikkeling van multipele cysten verspreid door de lever. PLD komt voor bij twee genetische aandoeningen: in combinatie met cystenieren bij autosomaal dominante polycysteuze nierziekte (ADPKD) en geïsoleerd in de lever bij autosomaal dominant polycysteuze leverziekte. Het natuurlijke beloop van PLD karakteriseert zich door een progressieve toename in aantal en grootte van levercysten. Dit kan resulteren in hepatomegalie. Een lever weegt normaliter zo'n 1.5 liter. Bij patiënten met ernstige PLD kan de lever wel 5-10 keer zo groot worden. Deze patiënten kunnen dan klachten ontwikkelen zoals buikpijn, snel een vol gevoel na het eten en kortademigheid. De leverfunctie in patiënten met PLD blijft intact.

Voor patiënten met symptomatische PLD bestaan er verschillende behandelingsmogelijkheden. Aspiratie sclerotherapie is een behandeling waarbij een symptomatische dominante cyste leeg wordt gezogen en alcohol wordt ingespoten om te zorgen dat de cyste verdwijnt. De chirurgische opties zijn fenestratie, resectie of levertransplantatie. Fenestratie kan worden uitgevoerd wanneer patiënten meerdere grote cysten hebben. De cysten worden dan operatief verwijderd. Bij een leverresectie wordt een deel van de lever verwijderd. Dit kan alleen wanneer de cysten zich met name in één deel van de lever bevinden en er voldoende leverweefsel zonder cysten overblijft. Bovenstaande therapieën zijn invasief en hebben een hoog risico op complicaties en recidieven. Een levertransplantatie is de enige genezende behandeling. Doordat ook dit een invasieve procedure is en donoren schaars zijn, wordt deze therapie weinig toegepast bij patiënten met PLD.

Omdat de huidige therapieën invasief zijn worden deze met name toegepast bij symptomatische patiënten of in patiënten die een grote kans hebben op het ontwikkelen van klachten. Op dit moment zijn we niet in staat te voorspellen welke patiënten symptomatische PLD ontwikkelen. Daarnaast is de relatie tussen het volume van de lever en symptomen niet geheel duidelijk. Tevens zijn er geen richtlijnen voor het starten van therapie. De vraag is dan ook welke factoren een rol spelen bij het initiëren van behandeling en de specifieke keuze voor een van de bovengenoemde behandelingsstrategieën. Tenslotte is er behoefte aan een niet-invasieve behandeling die het natuurlijk beloop van PLD kan beïnvloeden en klachten kan verminderen of voorkomen. In deze thesis worden verschillende van deze vraagstukken besproken.

Voorgaand onderzoek heeft laten zien dat er verschillende risicofactoren zijn voor het ontwikkelen van symptomatische PLD. In mijn proefschrift heb ik een overzicht gegeven van deze risicofactoren (**hoofdstuk 2**).

Logischerwijs denkt men dat levergrootte in verband staat met de klachten die patiënten ervaren. Echter, het bewijs hiervoor is niet eenduidig. Ook de rol van cystenieren hierin is onduidelijk. In dit proefschrift hebben we het onderzoek beschreven de relatie tussen het

gecombineerde lever- en niervolume, en symptomen onderzocht (**hoofdstuk 3**). We hebben dit onderzoek uitgevoerd bij patiënten met ADPKD omdat dit de grootste groep patiënten is waarbij PLD voorkomt. De resultaten lieten zien dat de combinatie van lever- en niervolume geassocieerd is met zowel gastrointestinale klachten, als pijn. Dit was met name te wijten aan het levervolume en er was geen aantoonbare relatie tussen niervolume en klachten. Deze bevindingen impliceren dat artsen bij patiënten met symptomatische ADPKD een behandeling moeten inzetten die zich richt op het reduceren van het levervolume.

Helaas bestaat er geen richtlijn die artsen helpt bij de keuze van een behandeling voor patiënten met PLD. Om te onderzoeken welke factoren een rol spelen bij het starten van behandeling en de keuze voor een specifieke behandelingsstrategie, hebben we een internationale database opgezet. Allereerst hebben we literatuuronderzoek gedaan om na te gaan aan welke eigenschappen een succesvolle database moet voldoen (**hoofdstuk 4**). Het onderzoek in de populatie opgenomen in mijn internationale database liet zien dat 35% van de patiënten afkomstige uit twee nationale tertiaire centra, worden behandeld met invasieve therapie (**hoofdstuk 5**). Het wel of niet ondergaan van een behandeling is onder andere afhankelijk van het centrum waar de patiënt terecht komt. De kans op behandeling neemt toe met het aantal symptomen en naarmate men langer de diagnose PLD heeft. Het verschil in behandeling tussen beide centra suggereert dat bewijs ontbreekt wat de beste behandeling is van een patiënt met PLD.

Aangezien de huidige behandelingen invasief zijn hebben we een internationale studie opgezet om na te gaan of ursodeoxycholzuur (ursochol) in staat was om het levervolume te reduceren in patiënten met PLD (**hoofdstuk 6a**). We hebben in totaal 34 patiënten met symptomatische hepatomegalie in deze studie geïncludeerd. De helft van deze patiënten werd behandeld met ursochol gedurende 24 weken en de andere helft kreeg geen behandeling. De resultaten van deze studie laten zien dat ursochol niet effectief was in het verkleinen van het levervolume (**hoofdstuk 6b**). Een subgroepanalyse in patiënten met ADPKD liet zien dat de levercysten bij hen minder hard groeiden in vergelijking met de levercysten in patiënten met ADPKD niet behandeld werden. Dit resultaat was niet zichtbaar in ADPLD patiënten. Op dit moment lijkt ursochol geen effectieve therapie voor het verkleinen van het levervolume in patiënten met PLD. Verder onderzoek moet uitwijzen of ADPKD en ADPLD patiënten verschillend reageren op ursochol.

De resultaten van mijn thesis laten zien dat levervolume een rol speelt in symptomatische ADPKD patiënten en dat behandeling gericht moet zijn op de lever. Huidige therapieën die het levervolume beïnvloeden zijn erg invasief en het behandelcentrum speelt een belangrijke rol in de keuze voor een specifieke behandelingsstrategie. Een niet-invasieve behandeling middels ursochol lijkt op dit moment niet effectief voor de behandeling van patiënten met symptomatische PLD.

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In 2004 ging ik studeren in het zuiden des Lands, Maastricht wel te verstaan. Gedurende het introductiekamp van gezondheidswetenschappen heb ik daar met een aantal studenten een spel mogen ontdekken, genaamd "stuiteren". U kent het wellicht niet, maar het spel stuiteren is een combinatie van volleybal en voetbal. Helaas voor ons is het stuiteren nog geen Olympische sport, maar een clublied hebben we al wel. Tijdens het stuiteren is de basis gelegd voor een hechte vriendengroep, onder de naam, u raadt het al "de Stuiters". Deze groep, bestaande uit 3 vrouwen en 4 mannen bevat mensen met passie, diepgang, humor en veel liefde. Met trots kan ik u vertellen dat ik daar deel van mag uitmaken. Ondanks dat we elkaar niet meer zo vaak zien als vroeger zorgt een avond of weekend met jullie altijd voor geweldige momenten en memorabele herinneringen. Lieve Stuiters, ik wil jullie bedanken voor onze vriendschap!

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Toen ik in het jaar 2011 verhuisde ben ik gaan hockeyen bij hockeyclub Den Bosch. In het jaar 2015 ben ik gewisseld van team en bij dames 4 gaan hockeyen. Lieve dames, ik ben ontzettend blij dat ik onderdeel ben van dit super leuke team. Door de positieve vibe die altijd binnen het team heerst krijg ik enorm veel energie en maakt het mij trots om er deel van uit te maken. Naast de fanatieke instelling maken we ook meer dan genoeg tijd vrij voor de 3^{e} helft. Dit blijkt vooral tijdens onze aanwezigheid bij de hockeydisco's en natuurlijk niet te vergeten het skiweekend!

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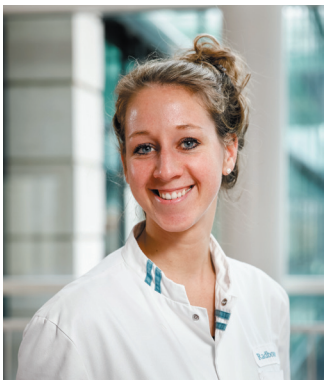
CURRICULUM VITAE

Hedwig Maria Antonia D'Agnolo was born on December the 19th, 1985 in Eindhoven, the Netherlands. She graduated from secondary school at the Lorentz Casimir Lyceum in Eindhoven and from 2004 until 2007 she studied Health Sciences at the University of Maastricht. In 2011 she finished her masters in medicine. From January 2013 to May 2016, Hedwig worked as a PhD student affiliated to the Division of Gastroenterology and Hepatology of Radboud University Medical Center (Promotor: prof. dr .J.P.H. Drenth, copromotor: dr. W. Kievit). She started her gastroenterology fellowship under supervision of dr. W. Smit (internal medicine, Jeroen Bosch Hospital, 's-Hertogenbosch), dr. P. Wahab (gastroenterology, Rijnstate Hospital Arnhem) and prof. dr. J.P.H. Drenth (Gastroenterology, Radboud University Medical Center, Nijmegen).



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2016 Lantinga, M.A., **D'Agnolo, H.M.A.**, Casteleijn, N.F., de Fijter, J.W., Meijer, E., Messchendorp, A.L., Peters, D.J.M., Salih, M., Spithoven, E., Soonawala, D., Visser, F.W., Wetzels, J.F., Zietse, B., Drenth, J.P.H., Gansevoort, R.T. Hepatic cyst infection during use of the somatostatin analogue lanreotide in autosomal dominant polycystic kidney disease: an interim analysis of the multicenter, open label, randomized DIPAK-1 study. Accepted for Drug Safety.

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ABSTRACTS

2016 The association of combined total kidney and liver volume with gastrointestinal symptoms and pain in patients with later stage ADPKD

2016 Ursodeoxycholic acid in advanced polycystic liver disease: an international multicenter randomized controlled phase 2 trial

2016 Polycystic liver disease patients with underlying diagnosis of polycystic kidney disease are more likely to undergo liver transplantation than patients with isolated polycystic liver disease

2015 Ursodeoxycholic acid as a volume reducing treatment for symptomatic polycystic liver disease: an international, multicenter, randomized controlled trial (CURSOR-trial)

AWARDS AND FUNDS

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